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AN INVESTIGATION INTO THE PATHOPHYSIOLOGY OF BREAST CANCER-RELATED LYMPHOEDEMA

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**AN INVESTIGATION INTO THE PATHOPHYSIOLOGY
OF BREAST CANCER-RELATED LYMPHOEDEMA**

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A thesis submitted to King's College London for the
degree of Doctor of Philosophy

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Abstract

Breast cancer-related lymphoedema (BCRL) is a chronic condition with associated physical and psychological sequelae. BCRL affects up to 25% of breast cancer patients, yet the aetiology is incompletely understood. The work described within this thesis will help further advance the understanding of the pathophysiology of BCRL, with a focus on whether patients are predisposed to developing BCRL.

Studies were conducted using qualitative and quantitative lymphoscintigraphy to assess the lymphatic system in breast cancer patients. The first study investigated muscle lymph flow in the upper limb. Lymphatic clearance rates were measured to investigate whether there was an abnormality in lymph flow prior to axillary lymph node surgery in patients who subsequently developed BCRL. Secondly, patients were assessed for the presence of upper limb lymphovenous communications to determine if these acted as a protective mechanism against the development of BCRL. Finally, in order to determine if there was a global dysfunction of the lymphatic system in patients previously treated for breast cancer, lower limb lymphatic function was assessed.

The first study demonstrated that those who went on to develop BCRL had a higher pre-operative muscle lymph flow compared with those who did not, indicating an underlying constitutional difference. The second study showed evidence of the presence of lymphovenous communications in several breast cancer patients studied, however the numbers were too small to show any correlation with the

development of BCRL. The final study showed that patients with BCRL had significantly impaired lower limb lymph flow compared with non-BCRL patients. Intriguingly, several non-BCRL patients were also found to have impaired lower lymph flow, raising the question of whether systemic treatment with chemotherapy was a significant contributory factor to this phenomenon.

In conclusion, these studies add evidence in support of the hypothesis that constitutional factors contribute to the development of BCRL.

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Abbreviations

ACOSOG	American College of Surgeons Oncology Group
ALMANAC	Axillary Lymphatic Mapping against Nodal Axillary Clearance
ALND	Axillary lymph node dissection
ALNT	Autologous lymph node transplantation
AMAROS	'After mapping of the Axilla: Radiotherapy or Surgery?'
ARM	Axillary reverse mapping
BCRL	Breast cancer-related lymphoedema
BCS	Breast-conserving surgery
BMI	Body mass index
BSUH	Brighton and Sussex University Hospitals NHS Trust
CDT	Complex decongestive therapy
DCIS	Ductal carcinoma in situ
DFS	Disease-free survival
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
EGFR	Epidermal growth factor receptor
EORTC	European Organisation for Research and Treatment of Cancer
ER	Oestrogen receptor
GSTT	Guy's and St Thomas' NHS Foundation Trust
HIG	Human immunoglobulin G
HER2	Human epidermal growth factor 2
IDC	Invasive ductal carcinoma no special type
k	The removal rate constant k (%/min)
LRR	Locoregional recurrence
LVA	Lymphaticovenous anastomoses
LVC	Lymphovenous communications
MLD	Manual lymphatic drainage
MRI	Magnetic resonance imaging
MRM	Modified radical mastectomy
Mx	Mastectomy
4NAS	Four-node axillary sampling
NAC	Neo-adjuvant chemotherapy
NSABP	National Surgical Adjuvant Breast and Bowel Project
OS	Overall survival
PR	Progesterone receptor
RBC	Red blood cell
ROI	Region of interest (gamma camera scans)
SLN	Sentinel lymph node
SLNB	Sentinel lymph node biopsy
^{99m}Tc	Technetium-99m
TNBC	Triple negative breast cancer
WHO	World Health Organisation
WLE	Wide local excision
VEGF	Vascular endothelial growth factor

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CHAPTER 1

Breast cancer

Breast cancer is the most common cancer in the UK, with approximately 50,000 new cases of breast cancer diagnosed each year (www.cancerresearchuk.org). It is the leading cause of cancer death globally in women.¹ The lifetime risk of developing breast cancer in the UK and USA is currently 1 in 8.²

1.1 Classification of breast cancer

Breast cancer is a heterogeneous disease, which consists of several subtypes with distinctive molecular features and clinical characteristics. Patient age, tumour size, tumour grade, lymph node status, lymphovascular invasion and receptor status are the major factors considered when assessing prognosis and determining the most suitable treatment for breast cancer patients.³

1.1.1 Histopathology

Breast cancer is divided into non-invasive and invasive cancer. Non-invasive cancer (carcinoma *in situ*) is a proliferation of epithelial cells that have not breached the basement membrane and myoepithelial layer. Ductal carcinoma *in situ* (DCIS) is the most common form of *in situ* disease, comprising approximately 85% of non-invasive disease. It usually involves a single duct system and its microscopic appearance is very variable. It is considered a true precursor of invasive breast cancer. It is classified into high, intermediate and low-grade categories, which

differ in aggression and potential for subsequent development of invasive disease. Non-invasive 'lobular' proliferative changes are divided into lobular carcinoma *in situ* (LCIS) and atypical lobular hyperplasia (ALH). LCIS is a more extensive form of ALH, which has the potential to progress to invasive carcinoma.^{4,5}

Histological criteria and immunohistological (IHC) analysis is performed on tumour specimens. The World Health Organisation (WHO) classification describes 18 distinct histological types of invasive cancer.⁶ Invasive ductal carcinoma, not otherwise specified, also known as no special type (NST), is the most common and accounts for 70-80% of all breast cancers. Other breast cancer types include lobular carcinoma (10-15% of cases), medullary (5%), mucinous (2%) and tubular carcinoma (1%).² In addition to the histological type, tumour grade (an assessment of differentiation and proliferative activity), tumour size and receptor status are collected. The classification of different subtypes helps guide therapy and has been valuable for prognostication.⁷

1.1.2 Receptor status

Breast tumours may express receptors of which the three most important are oestrogen (ER), progesterone (PR) and human epidermal growth factor 2 (HER2). Oestrogens stimulate breast tumour proliferation and 60-70% of breast cancers are ER-positive.⁸ ER-positive tumour patients have a lower risk of mortality than ER-negative patients. The NSABP 06 trial found improved disease free survival (DFS) and overall survival (OS) in ER positive patients.⁹ The Survival, Epidemiology and End Results (SEER) programme analysed data from 155,175 breast cancer patients

with known receptor status. Patients were categorised as ER+/PR+ (63%), ER+/PR- (13%), ER-/PR+ (3%) and ER-/PR- (21%). There was a higher relative risk of morbidity comparing ER+/PR+ patients to all other patients across the majority of other tumour characteristics (tumour size, grade, stage and number of positive nodes). ER negativity appeared to be a greater determinant of morbidity compared to PR negativity.¹⁰ Although this study was limited due to differing assays and techniques for determining receptor positivity and absence of full adjuvant hormonal therapy and chemotherapy, other studies have confirmed better survival in patients with ER+ tumours (Table 1).

	Number of patients	Follow-up (years)	Five year overall survival (%)	
			ER positive	ER negative
Survival, epidemiology and end results (SEER) database ¹⁰	155,175	8	92	81
Danish breast cancer cooperative group 89 & 99 ¹¹	26,944	5	85	69
NSABP 06 ⁹	1157	5	92	82

Table 1 5-year overall survival in prospective studies comparing oestrogen (ER) positive and negative patients

NSABP, National Surgical Adjuvant Breast and Bowel Project; ER, oestrogen receptor

HER2 is part of the epidermal growth factor receptor (EGFR) family and is overexpressed in 15-20% of breast cancers.¹² There is a significant correlation between HER2 overexpression and poorer prognosis, with decreased DFS and OS in node-positive patients.¹³ In a systematic review by Mirza *et al*, HER2 overexpression showed independent prognostic significance in node-negative disease.¹⁴ However,

at present there is no consensus on the association between HER2 status and other prognostic factors (e.g. tumour size, lymphovascular invasion and response to hormonal therapy). There has been a lack in standardisation of assay methodology, which has contributed to conflicting conclusions from older studies.

Tumours that do not express ER, PR or HER2 are called triple-negative breast cancers (TNBC), and account for approximately 15% of all breast cancers. Although described as one group, TNBCs consist of a heterogeneous group of different tumour types. TNBC patients tend to be younger, have larger tumours at presentation, increased nodal positivity, higher tumour grade and a poorer prognosis. There is a significantly lower OS and DFS up to 5 years from diagnosis. There is a rapid rise in recurrence rates in the first 1-3 years, with a shorter time from distant recurrence to progression and death. However, after 10 years, TNBC patients are less likely to relapse than ER positive patients, suggesting a more aggressive but potentially curable entity.^{15,16} Receptor status is used in the selection of appropriate systemic therapy (sections 1.2.4.2 and 1.2.4.3).

1.1.3 Molecular classifications

Gene expression profiling has allowed a move towards molecular profiling of breast cancer. Seminal work by Perou *et al* in 2000 classified breast cancer based on gene expression profiling, describing four molecular subtypes: luminal, HER2 overexpression, basal-like and normal breast tissue-like.¹⁷ Further work has found subgroups and other subtypes and there are currently six recognised subtypes: luminal A, luminal B, HER2 overexpression, basal-like, normal breast tissue-like and

claudin-low.¹⁸ Microarray-based gene expression profiling has been used to predict the outcomes for patients, and it is the proliferation-related component of prognostic signatures that predict the outcome.¹⁹ Mammaprint (70-gene signature, Amsterdam) and Oncotype DX (polymerase chain reaction-based assay of 21 genes) are examples of platforms that have been approved for clinical application to predict disease outcome. They also help determine which patients might benefit from chemotherapy due to the correlation of chemo-sensitivity and the genetic profile of certain breast tumours.^{20,21}

Molecular taxonomy is constantly evolving and is still a work in progress. Gene expression has led to an improved understanding of signalling pathways and has allowed the development of targeted therapies, with the aim of a more personalised approach to breast cancer treatment.

1.2 Management of breast cancer

1.2.1 Diagnosis

Breast cancer diagnosis is based on a multi-disciplinary ‘triple assessment’ approach. This comprises clinical assessment, imaging and histopathological assessment.

Clinical assessment includes taking a detailed history and examination. The main imaging techniques used are mammography, ultrasound (US) and magnetic resonance imaging (MRI). Mammography is the most commonly used modality to

image breast cancer and can identify changes in breast density and calcifications. This is less useful in patients with dense breasts. The overall sensitivity and specificity has been estimated to be 60-95%.²² Ultrasound imaging is generally used in addition to mammography in patients with dense breasts.

Magnetic resonance imaging is the most sensitive imaging modality for detecting and staging breast cancer. It is more sensitive than other modalities in the assessment and detection of multicentric/multifocal disease, especially in cases of lobular carcinoma.^{23,24} However, MRI has been shown to overestimate the tumour size and has limited availability as well as increased cost compared to mammography and US.^{25,26} The aim of breast conserving surgery (BCS) is to completely excise the tumour and obtain clear margins. A lower re-excision rate is beneficial to both patients and healthcare resources. There is limited evidence from randomised control trials (RCTs) for the use of MRI in pre-operative imaging and planning surgery for breast cancer. Many studies were non-randomised and retrospective with inconsistent methodology.

Table 2 summarises the two main RCTs for routine MRI use in breast cancer; the COMICE and MONET trials, and two of the largest observational studies.²⁷⁻³⁰ They concluded that routine MRI does not decrease the re-excision rate following wide local excision. Counter-intuitively, MRI in non-palpable tumours was actually found to significantly increase the re-excision rate, which the authors were not able to fully explain. There is more evidence to support MRI in patients with lobular cancer

and those at high risk of cancers. In 2010 the European Society of Breast Cancer Specialists (EUSOMA) group evaluated the available evidence and reached a consensus for use of MRI in all breast cancer patients. Plana *et al* conducted a more recent systematic review and meta-analysis, with similar conclusions to the EUSOMA group.²⁵ The key recommendations are summarised in Table 3 with the corresponding levels of evidence.

Once imaging techniques have identified a suspicious lesion, samples of cells/tissue are required to confirm the diagnosis. Patients have either core biopsy or fine-needle aspiration cytology (FNAC) of suspicious breast tissue or axillary nodes. This is usually performed using US or X-ray guidance. Core biopsy is the gold standard for tissue diagnosis.^{31,32}

Once the diagnosis has been confirmed, the triple assessment findings are usually discussed and reviewed by a multidisciplinary panel. Treatment options are then discussed with the patient and a suitable management plan formulated.

Authors	Year	Type of study	Number of patients	Additional pre-operative imaging		Re-excision rate		Significance <i>p</i>	Conclusion
				MRI	No MRI	MRI group	No-MRI group		
<i>Turnbull et al COMICE trial</i> ²⁷	2010	RCT – palpable breast cancers	1623	816	807	19%	19%	0.77	No benefit with additional MRI imaging
<i>Peters et al MONET trial</i> ²⁹	2011	RCT – non-palpable breast cancers	418	207	211	34%	12%	0.008	Significantly higher re-excision rate in patients having MRI
<i>Pengel et al</i> ²⁸	2009	Retrospective comparative cohort	349	173	176	13.8%	19.4%	0.17	No significant difference in re-excision rate
<i>Bleicher et al</i> ³⁰	2009	Retrospective observational	577	130	447	21.6%	13.8%	0.20	No significant difference in re-excision rate

Table 2 Main trials investigating potential benefit of additional MRI scanning in pre-operative assessment of breast cancer patients

MRI, magnetic resonance imaging; RCT; randomised control trial;

Recommendations for MRI	Level of evidence
Pre-operative MRI in newly diagnosed lobular cancer	2a
Pre-operative MRI for patients aged > 60 years with > 1cm discrepancy in size between mammogram and ultrasound	2b
Verification of pre-operative MRI findings with percutaneous biopsy	EPO
Any changes to therapeutic planning resulting from pre-operative MRI findings should be decided by MDT	EPO
High risk patients; annual MRI offered to: <ul style="list-style-type: none"> BRCA1, BRCA2 and TP53 mutation carriers 1st degree relatives with >50% risk for BRCA1, BRCA2 and TP53 mutation previous mantle radiotherapy patients patients inconclusively tested for BRCA mutation with > 20-30% lifetime risk patients undergoing prophylactic mastectomy to screen for occult breast cancer 	EPO EPO 3 2 EPO
Patients due to have NAC with potentially operable large tumours should have pre-chemotherapy MRI	1
Post NAC patients for measurement of residual disease. This should be > 2weeks after last NAC cycle and < 2 weeks before surgery	EPO

Table 3 EUSOMA key recommendations for MRI²⁴

MRI, magnetic resonance imaging; MDT, multi-disciplinary team; NAC; neo-adjuvant chemotherapy; EPO, expert panel opinions

1.2.2 Surgery

The surgical management of breast cancer has seen significant changes over the past 150 years. Axillary lymph node dissection has been an integral part of breast cancer surgery for more than a century. Current surgical treatment of patients with invasive breast cancer includes excision of the primary tumour and axillary lymph node staging or clearance.

1.2.2.1 Surgery to the breast

In the late 19th century patients often presented with late stage breast cancer as social norms prevented women from seeking medical attention early.³³ Halsted

believed the major mechanism of tumour spread was by lymphatic permeation and advocated that extensive surgery was necessary to treat breast cancer. The radical mastectomy became the mainstay of treatment for breast cancer, which involved removal of the breast, lymph nodes and pectoralis muscles.³⁴ Halsted's surgical procedure had lower recurrence rates than any other series at the time and remained the gold standard of surgical treatment for the next 50 years (Table 4).

Surgeon	Year	Number of patients	Local recurrence rates
Billroth	1867 - 76	170	82%
Fischer	1871 – 78	147	75%
Volkman	1874 - 78	131	60%
Bergmann	1882 - 87	114	51-60%
Halsted	1889 - 94	50	6%

Table 4 Local recurrence rates for breast cancer in the 19th Century³⁴

The radical mastectomy was challenged in later years due to the advent of radiotherapy and further insight into human anatomy, dismissing lymphatic permeation as the major cause of tumour spread. In 1948 Patey and Dyson published studies that compared radical mastectomy with modified radical mastectomy (MRM), where the pectoralis minor was sacrificed but the pectoralis major was preserved. They found comparable survival and local recurrence rates (LRR), but with improved cosmesis and decreased blood loss in patients undergoing MRM. They showed that less extensive surgery was equally effective.^{35,36} Patey and Dyson also looked at the use of radiotherapy in combination with breast cancer surgery. The local recurrence rates were comparable but the side effects were high

in these patients, and they concluded that radiotherapy should not be routinely used until further clinical trials were conducted.³⁶

The National Surgical Adjuvant Breast and Bowel Project trial (NSABP B-04) compared outcomes of radical mastectomy and MRM, with and without radiotherapy, in the primary treatment of breast cancer. The results revolutionised breast cancer surgery by proving that radical mastectomy and its accompanying functional and cosmetic morbidity were unnecessary in terms of providing the patient with good overall and disease-free survival outcomes.³⁷

Current surgical options to treat breast cancer include modified radical mastectomy (with or without breast reconstruction) and breast conserving surgery (BCS). Mastectomy is performed in patients with large tumours (especially in women with small breasts), some centrally placed tumours involving the nipple and areolar, multicentric disease, associated extensive DCIS or positive margins after BCS despite one or two further re-excisions. Mastectomy rates are variable, both internationally and in the UK.³⁸ In the developed world, 25-30% of cancers are treated with mastectomy.³⁹ Nipple-sparing and skin-sparing mastectomies have raised concerns regarding local recurrence rates. These procedures are now thought to be oncologically safe in carefully selected patients, although longer-term studies are still needed.⁴⁰⁻⁴²

It was not until the 1970s that Veronesi *et al* conducted a randomised trial, which aimed to demonstrate that radiotherapy combined with breast conserving surgery achieved comparable results to radical mastectomy. The results showed similar overall and breast-specific survival rates in both groups.⁴³ In patients with stage I or II cancer, BCS with radiotherapy has become the treatment of choice, with several trials showing comparable results to mastectomy regarding LRR and overall survival.⁴⁴⁻⁴⁸ There were six RCTs which formed the basis of the National Institutes of Health (NIH) consensus statement recommending the increased use of BCS and radiotherapy (Institute Gustave-Roussy (IGR-Paris),⁴⁷ NSABP 06,⁴⁹ Milan-World Health Organisation,⁵⁰ European Organisation for the Research and Treatment of Cancer (EORTC) 10801,⁴⁸ Danish,⁵¹ and U.S. National Cancer Institute trials⁴⁶). The results of the pooled data from the main trials are summarised in Table 5. Breast screening programmes have significantly impacted the stage at which patients are diagnosed with cancer, with larger numbers of patients presenting at earlier stages who are suitable for BCS.⁵² The increased use of neo-adjuvant chemotherapy (NAC) and endocrine therapy to downstage tumour size has also increased the number of patients suitable for BCS. Approximately two-thirds of patients diagnosed with breast cancer currently undergo BCS as their initial breast surgery.⁵³⁻⁵⁵

	Number of trials	Begin date for trials	Node negative patients (n = 7287)		10 year LRR or distant recurrence (%)		Absolute % reduction with radiotherapy
			BCS + radiotherapy	BCS	BCS + radiotherapy	BCS	
Original lumpectomy trials ¹	6	1976 – 1986	1223	1197	27.8	47.9	20.1
Sector resection or quadrantectomy ²	4	1981 – 1991	986	970	14.3	25.9	11.6
Lumpectomy in low risk patients ³	7	1989 - 1999	3675	3612	15.6	31.0	13.6

Table 5 10-year locoregional recurrence (LRR) and distant recurrence pooled data from randomised trials of patients undergoing breast-conserving surgery (BCS) with or without radiotherapy⁵⁶

(¹ NSABP 06, St. George's, Ontario COG, Scottish, West Midlands, CRC UK, ² Uppsala-Orebro, Int Milan III, Tampere, SweBCG 91-RT, ³ NSABP B21, GBSG V Germany, BASO II, CALGB 9343, ABCSG 8a, PRIME trials)

1.2.2.2 Surgery to the axilla

Histopathological examination of lymph nodes removed during surgery provides accurate prognostic information and helps determine the most appropriate adjuvant therapy. Axillary surgery is also therapeutic by removing lymph nodes containing metastatic disease thereby decreasing the risk of axillary nodal recurrence. It is estimated that 30-40% of early breast cancer patients have axillary lymph nodal involvement.⁵⁷ Surgical options for the axilla include axillary lymph node dissection (ALND), four-node axillary sampling (4NAS), blue dye assisted four-node axillary sampling and sentinel lymph node biopsy (SLNB).⁵⁸⁻⁶²

Axillary lymph node dissection was previously the standard approach for axillary lymph node surgery. It can be performed to level one, two or three, based on the anatomical relationship of the axillary nodes to pectoralis minor. ALND is associated with significant morbidity, e.g. seroma formation, limited upper limb and shoulder mobility, sensory loss and lymphoedema. In patients in whom there is no axillary nodal involvement, these complications significantly affect quality of life without an associated clinical benefit. As a result alternative methods were sought to reduce the morbidity of this procedure in such patients.⁶³

In most cases, lymphatic spread of cancer from the breast to the axillary nodes is systematic from levels 1 to 3 with skip metastases occurring infrequently.^{64,65} The sentinel node (SLN) is the first node(s) to receive lymph from the site of the tumour

and should be the first node to be involved if there is metastatic spread. Hence an alternative method of staging the axilla is SLNB.

The first SLNB was performed in 1951 by Gould during a parotidectomy.⁶⁶ The technique was then used in penile cancer by Cabanas in the 1970s.⁶⁷ Morton *et al* adapted the procedure for cutaneous melanoma, which was presented at a World Health Organisation conference for melanoma in 1989. This is believed to be the turning point when SLNB was accepted by the surgical community.⁶³ In 1994, Guiliano and colleagues introduced SLNB into the management of breast cancer patients. They reported accurate predicted nodal status in 96% of patients in whom blue dye mapping identified the sentinel nodes.⁶⁸ Krag *et al* investigated the use of radioisotopes and gamma probe for localisation of the SLN and then collaborated with the National Cancer Institute to develop clinical guidelines, which are still widely used.⁶⁹⁻⁷¹ Further work regarding localisation techniques was done by McMasters *et al* to establish whether blue dye, radioisotope or a combination of the two was superior. They concluded that a combination of blue dye and radioisotope gave the highest identification rate of the SLN with the lowest false negative rate.⁷² The same group conducted a multicentre study looking at the best method of radioisotope injection, and concluded that intradermal injection rather than peritumoural or subdermal injection was superior in identification of the SLN.⁷³ The SLN(s) is identified by injection of a radio-tracer and blue dye into the dermis of the periareolar region. When the axilla is surgically exposed, visual inspection and a hand-held gamma probe allows identification of the nodes that

have taken up tracer and dye and nodes that are radioactive and/or blue are subsequently removed.

The NSABP B32 was one of the largest prospective trials that compared ALND with SLNB in a group of 5611 breast cancer patients. The results showed equivalence in the two groups for overall survival (OS), disease-free survival (DFS) and regional control, and concluded that SLNB was appropriate, safe and effective in patients with node negative disease.⁷⁴ SLNB has been validated in other prospective, multicentre, international trials and long-term DFS and OS are summarised in Table 6. SLNB is the current recommended standard of care for a clinically and radiologically node negative axilla.⁷⁴⁻⁷⁷

Newer technologies have allowed more detailed examination of the sentinel node including serial sectioning haematoxylin & eosin (H&E) staining, polymerase chain reaction (PCR) and immunohistochemistry (IHC). SLN involvement is staged according to the American Joint Committee on Cancer (AJCC) classification. Tumour deposits < 0.2 mm are referred to as isolated tumour cells (ITCs). Micrometastases refer to deposits > 0.2 mm but < 2mm. Tumour deposits > 2 mm are referred to as macrometastases.⁷⁸ Patients with ITCs are considered node negative and those with micro- or macrometastases node positive. If the SLN is found to be tumour-free, this would indicate that the rest of the axillary nodes do not contain metastases^{79,80} and patients do not need to undergo further axillary treatment.⁸¹ If the SLN is

found to be positive, then patients usually progress to have completion ALND, which takes place in approximately 50% of patients undergoing SLNB.^{76,79}

Controversy surrounding this approach remains and it is argued that selected early-stage breast cancer patients receiving adjuvant therapy may not require completion axillary lymph node dissection (cALND) for regional control.^{75,82}

The American Society of Clinical Oncologists (ASCO) guidelines (2005) reported further axillary involvement in 20-35% of patients with micrometastases in the SLN, recommending completion ALND (cALND) in this group.^{83,84} This has been challenged by other studies reporting low axillary recurrence rates of 0 - 3.7% in patients with micrometastatic disease in SLNs with follow-up periods ranging from 30 to 60 months.^{85,86} The International Breast Cancer Study Group (IBCSG) trial 23-01 randomised patients with micrometastases in SLNs into two groups; cALND and no further surgery. This study demonstrated a 2% local recurrence rate in the no further surgery group and comparable rates of disease-free survival and overall survival at 5 years.⁸⁷ The Agency for Health Technology Assessment and Research (AATRM) 048/13/200 conducted a multicentre randomised controlled trial comparing patients with SLN micrometastatic disease who underwent cALND (control group) with those who did not (study group). This study found no significant difference in 5-year disease-free survival between the groups and reported an axillary recurrence rate of 1% in the control group and 2.5% in the study group after a median follow-up interval of 62 months.⁸⁸

	Recruitment years	Number of patients	Follow-up	SLNB* (95% CI) %	ALND** (95% CI) %
Zavagno et al ⁷⁷	1999-2004	697	5-year DFS OS	87.6 (83.3-90.9) 94.8 (91.6-96.8)	89.9 (85.3-93.1) 95.5 (92.2-97.5)
Krag et al ⁷⁴	1999-2004	5611	8-year DFS OS	81.5 (79.6-83.4) 90.3 (88.8-91.8)	82.4 (80.5-84.4) 91.8 (90.4-93.3)
Veronesi et al ⁸⁹	1998-1999	516	10-year DFS OS	89.9 (85.9-93.9) 93.5 (90.3-96.8)	88.8 (84.6-92.9) 89.7 (85.5-93.8)

Table 6 Long term disease free survival (DFS) and overall survival (OS) in randomised controlled trials validating SLNB

*SLNB followed by ALND in node positive patients;** SLNB followed by ALND; CI, confidence interval

The American College of Surgeons Oncology Group (ACOSOG) Z0011 trial assessed the local and regional recurrence in patients with positive SLNB, comparing patients who were randomised to cALND with those who had no further surgery. The trial concluded similar, low 5-year LRR in patients who underwent cALND and those who did not (3.1% vs. 1.6% respectively). Regional recurrence rates were similarly very low with 0.5% in cALND group and 0.9% in SLNB group. This was a pivotal trial, but there were several limitations that could have potentially biased the results. Firstly, the study aimed to recruit 1900 patients, but was stopped early due to low accrual and event rates and only 891 patients were randomised, with 813 patients receiving treatment. The patients recruited were clinically node negative stage one or two patients and treated with BCS. This excluded mastectomy patients and those with stage III disease, so the patient group was not representative of patients with more widespread/aggressive disease, who would have been more likely to have axillary nodal involvement. Approximately 45% of the patients in the SLNB group had micrometastases, which also indicates minimal axillary involvement and is associated with a lower local recurrence rate. All patients had opposing tangential field whole breast irradiation, which included treatment to level 1 and some of level 2 nodes in the axilla. Lastly, over 95% of all patients had adjuvant systemic therapy, with just below 60% receiving chemotherapy, which is also known to decrease the local recurrence rates.⁷⁵ This may not be wholly reflective of adjuvant systemic treatment in patients with similar clinical presentation in other units, which would potentially make the results of this trial less pertinent.

Glechner *et al* (2013) conducted a systematic review and meta-analysis of SLNB only versus cALND in patients with early invasive breast cancer and positive SLNB. The meta-analysis included 50,120 patients and they found similar 5-year overall survival and LRR in the two groups, with higher quality of life (QoL) in SLNB only patients. They suggested that for women with early invasive breast cancer (T1 or T2 disease) undergoing BCS with radiotherapy and systemic therapy, SLNB alone was an option that could be discussed with the patient as an alternative to completion ALND.⁹⁰

Four node axillary sampling (4NAS) is a procedure that involves the removal of four palpably enlarged axillary lymph nodes and examining them for evidence of metastatic disease.⁹¹ Chetty *et al* conducted a RCT of 466 patients, randomising patients undergoing BCS to ALND or 4NAS, with selective use of axillary radiotherapy in patients undergoing 4NAS. They reported no difference in DFS and OS (median follow-up 4.1 years), and no difference in time to axillary or breast recurrence ($p = 0.94$ and 0.97 , respectively).⁹² Blue dye assisted 4NAS is a technique that is a targeted four node sampling assisted with blue dye. In the era of SLNB and blue-dye, perhaps the use of 4NAS no longer has a place, although this technique may be an acceptable and cost-effective method for staging the axilla in the absence of radioisotope facilities.

1.2.3 Radiotherapy

Patients may have radiotherapy administered after breast conserving surgery or mastectomy.⁵⁶ The Early Breast Cancer Trialists Collaborative Group (EBCTCG)

publish 5-yearly updates of randomised trials of radiation for breast-conserving surgery and mastectomy. Table 5 summarises the results from the 2011 update of the main randomised trials regarding 10-year locoregional or distant recurrence in node negative breast cancer patients undergoing BCS with or without radiotherapy.⁵⁶ In the context of BCS, radiotherapy to the conserved breast halves the local recurrence rate and decreases breast cancer-related deaths by a sixth.⁵⁶ Post-mastectomy radiotherapy (PMRT) to the chest wall is recommended if there is thought to be a high risk of locoregional recurrence, i.e. ≥ 4 positive axillary lymph nodes, T3/T4 lesions or invasion of skin or underlying muscle.⁹³⁻⁹⁵ Patients usually receive external beam radiotherapy (EBRT) to the breast or chest wall, with a dose of 40 Gray in 15 fractions.⁸¹ The Danish and British Columbia randomised trials compared LRR, DFS and OS in post-mastectomy women undergoing radiotherapy in addition to adjuvant tamoxifen or chemotherapy. Patients having radiotherapy in addition to adjuvant treatment had a lower LRR rate compared with several other non-randomised series.⁹⁶⁻⁹⁸ They reported a locoregional recurrence relative risk reduction of approximately two-thirds (Table 7). The reduction in 10-year overall survival was reported as 9%, but the impact of PMRT on overall survival has been debated. There has been a change in adjuvant systemic treatment since these trials recruited and their results may not be translatable to current practice. The use of PMRT in the intermediate-risk groups remains controversial. The SUPREMO trial closed recruitment in 2013 and is aiming to investigate the role of PMRT in intermediate-risk breast cancer patients.⁹⁹

	Years of recruitment	Treatment after breast cancer surgery (n)		Locoregional recurrence rate (%)		Disease free survival (%)		Overall survival (%)	
		Chemotherapy + PMRT	Chemotherapy	Chemotherapy + PMRT	Chemotherapy	Chemotherapy + PMRT	Chemotherapy	Chemotherapy + PMRT	Chemotherapy
Danish breast cancer cooperative group 82b. 10 year results ⁹⁷	1982 – 1989	852	856	9	32	48	34	54	45
British Columbia randomised trial. 20 year results ⁹⁸	1979 - 1986	164	154	10	26	48	31	47	37

Table 7 Results from Danish and British Columbia trials comparing locoregional recurrence; disease-free survival and overall survival in pre-menopausal women receiving chemotherapy with or without post-mastectomy radiotherapy (PMRT)

	Years of recruitment	Treatment after breast cancer surgery (n)		Locoregional recurrence rate (%)		Disease free survival (%)		Overall survival (%)	
		Tamoxifen + PMRT	Tamoxifen	Tamoxifen + PMRT	Tamoxifen	Tamoxifen + PMRT	Tamoxifen	Tamoxifen + PMRT	Tamoxifen
Danish breast cancer cooperative group 82c. 10 year results ⁹⁶	1982 - 1990	686	689	7	36	36	24	45	36

Table 8 Results from the Danish trial comparing locoregional recurrence; disease-free survival and overall survival in post-menopausal women receiving tamoxifen with or without post-mastectomy radiotherapy (PMRT)

Standard EBRT requires daily radiation for a period of at least 3 weeks, which can be a burden to patients and on healthcare resources. There have been trials evaluating an alternative, more conservative radiotherapy technique with accelerated partial breast irradiation (PBI) in intraoperative radiotherapy (IORT). TARGIT-A was a randomised, non-inferiority trial comparing single-dose targeted IORT (TARGIT) with whole breast EBRT in patients with invasive ductal carcinoma (NST). There were significantly fewer non-breast cancer deaths in the TARGIT group and no difference in breast cancer mortality or wound-related complications. However, there was a significant increase in 5-year LRR in the TARGIT group compared with the EBRT group (3.3% vs. 1.3%, $p = 0.04$).¹⁰⁰ The ELIOT trial similarly randomised early breast cancer patients to IORT and whole breast EBRT. They found a significantly higher LRR after a median follow-up of 5.8 years of 4.4% in the IORT group compared with 0.4% in the EBRT group ($p < 0.0001$).¹⁰¹ PBI is not recommended outside of clinical trials and it should remain investigational until more evidence for its safety and efficacy has been evaluated.

Patients may be given radiotherapy to the axillary or supraclavicular fossa (SCF) nodes. Patients with negative sentinel nodes or those who have undergone ALND do not require radiotherapy to the axilla. Radiotherapy has been thought to be potentially less invasive than completion ALND in patients who are found to have positive SLNs, but it was not known if this would be more effective. The 'After mapping of the Axilla: Radiotherapy or Surgery?' (AMAROS) trial from the European Organisation for Research and Treatment of Cancer (EORTC), enrolled patients with

positive SLNs and then randomised them to either undergo cALND or axillary nodal irradiation.¹⁰² Results showed that the local recurrence rate was very low in both groups with rates of 0.43% in patients undergoing ALND and 1.19% in those undergoing axillary radiotherapy, with a median follow-up of 6.1 years. The overall survival and disease-free survival was not significantly different in either group (OS; 93.3% ALND patients and 92.5% axillary radiotherapy, DFS: 86.9% ALND and 82.6% axillary radiotherapy).¹⁰² SCF recurrence is more common in patients with heavily node positive axillae. The SCF should be irradiated in patients with 4 or more nodes involved to decrease the morbidity associated with SCF recurrence.^{81,103}

1.2.4 Systemic therapy

Chemotherapy, endocrine therapy and biologically targeted therapies have contributed to a marked decrease in recurrence and mortality from breast cancer. Patients can receive a combination of some or all of these treatments in both the adjuvant and neo-adjuvant settings.

1.2.4.1 Chemotherapy

Chemotherapy plays an essential role in the adjuvant and neo-adjuvant treatment of intermediate and high-risk breast cancer patients.¹⁰⁴ In the 1970s the Milan group demonstrated that breast cancer recurrence could be reduced by the addition of adjuvant chemotherapy, using CMF (cyclophosphamide, methotrexate and 5-fluorouracil).¹⁰⁵ Anthracycline-containing regimens were investigated by the NSABP in the 1990s, with the aim of reducing the duration of treatment, the number of hospital visits and morbidity. The results of the NSABP B-15 trial

concluded that the results for CMF and AC (doxorubicin and cyclophosphamide) were equivalent.¹⁰⁶ AC became the 'gold-standard' at that time. Over the following years, CMF and AC became the standards against which other regimens were compared. Taxanes (paclitaxel and docetaxel) were developed in the 1980s and initially used in metastatic breast cancer. Henderson *et al* found that AC followed by paclitaxel was more effective than AC alone.¹⁰⁷ The Breast Cancer International Research Groups (BCIRG) -001 trial replaced 5-fluorouracil in FAC with docetaxel (T), and the results showed that TAC was more effective than FAC in node positive patients.^{107,108} The French Adjuvant group modified this regimen further, substituting doxorubicin for epirubicin, and following three cycles of FEC with three cycles of docetaxel.¹⁰⁹ FEC-T is now a commonly used regimen for patients with positive axillary lymph nodes in the UK. The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) was established in 1985 to co-ordinate the meta-analyses of randomised trials of patients receiving adjuvant treatment. Although there is no one gold standard chemotherapy regimen, the EBCTCG has drawn some important conclusions. Treatment with CMF or 4AC (4 cycles of doxorubicin and cyclophosphamide) has been found to be approximately equivalent, with a relative reduction of breast cancer mortality rates by 20-25%. Also, chemotherapy agents given in addition to 4AC were more effective than standard regimens, e.g. addition of taxanes, with a further proportional reduction of 15-20% in mortality rates. The EBCTCG concluded that the 10-year risk of death from breast cancer is reduced by about a third when comparing patients receiving effective chemotherapy compared with those who did not receive chemotherapy.¹¹⁰

Patients with locally advanced breast cancer may receive neo-adjuvant chemotherapy (NAC) to downstage primary operable tumours towards more conservative surgery or convert an unresectable, locally advanced tumour into an operable one.¹¹¹ NAC is as effective as adjuvant chemotherapy with regard to survival benefit in patients with locally advanced disease.¹¹² The regimens prescribed are similar to those used in the adjuvant setting. Patients are usually reassessed after 2-3 cycles of NAC and if the tumour is responding, then chemotherapy is continued for a total of 6-8 cycles.¹¹³ The key trials comparing NAC and adjuvant chemotherapy are summarised in Table 9. Patients showing a pathological complete response (pCR) demonstrate improved overall survival,¹¹⁴ and this is more likely in ER-negative than ER-positive tumours.¹¹⁵ Odds of pCR were highest for the triple negative and HER2+/hormone receptor negative subtypes, with evidence of an influential effect on achieving pCR in the latter subtype through inclusion of HER2-directed therapy with NAC.¹¹⁶ However, if the tumour does not show improvement or shows progression despite NAC, then patients should proceed directly to surgery.

Chemotherapy is the mainstay of systemic treatment for TNBC, but there is currently no standard chemotherapy regimen. Some TNBC patients show a pCR after NAC, but on the whole, the TNBC group has a worse outcome after chemotherapy than hormone receptor positive patients.¹⁶

There are significant toxicities associated with chemotherapeutic drugs, which can cause both long and short-term mortality and morbidity. Side effects include nausea, vomiting, myelo-suppression, cardiotoxicity and secondary malignancy.⁶² The vast heterogeneity in breast cancer means it is difficult to predict how patients will respond to different regimens. The benefits and harm of chemotherapy need to be determined by weighing the risk of future relapse against co-existing co-morbidities, thereby tailoring treatment as much as possible.

	Recruitment years	Number of patients	Chemotherapy regimen	Follow-up (months)	Local Regional Recurrence (%)		Overall Survival (%)	
					NAC	Adjuvant	NAC	Adjuvant
NSABP B18 ¹¹⁷	1988-1993	1523	AC	114	15	13	69	70
ECTO ¹¹⁸	1996-2002	1355	AT & CMF	76	4.6	4.1	84	82-85
EORTC 10902 ¹¹⁹	1991-1999	698	FEC	120	14	13	65	66
Institut Curie ¹²⁰	1986-1990	414	FAC	105	27	19	65	60

Table 9 Summary of randomised controlled trials in patients receiving neo-adjuvant and adjuvant chemotherapy

NSABP, National Surgical Adjuvant Breast and Bowel Project; ECTO, European cooperative trial in operable breast cancer; EORTC, European Organization for Research and Treatment of Cancer; NAC, neo-adjuvant chemotherapy; AC, doxorubicin, cyclophosphamide; AT, doxorubicin, paclitaxel; CMF, cyclophosphamide, methotrexate, fluorouracil; FEC, fluorouracil, epirubicin, cyclophosphamide; FAC, fluorouracil, doxorubicin, cyclophosphamide;

1.2.4.2 Endocrine therapy

Endocrine therapy aims to prevent the growth stimulation effects of oestrogen signalling in breast cancer. Tamoxifen was first discovered in the 1950s and initially assessed as a contraceptive. Tamoxifen acts by binding to the oestrogen receptor and inhibiting the expression of oestrogen-regulated genes, which are essential for tumour growth in oestrogen-dependent tumours. It was initially used in post-menopausal women with advanced breast cancer. The NSABP trials assessed progression-free survival in pre- and post-menopausal women with early breast cancer and positive results led to tamoxifen being the gold standard for women with ER positive breast cancer.¹²¹ A recent meta-analysis by the EBCTCG has concluded that a five-year course of tamoxifen reduces the 15-year risk of breast cancer recurrence and mortality by approximately a third. The reduction was greater in patients with strongly ER-positive tumours compared with marginally ER-positive tumours.¹²² There have been trials investigating the advantage of long-term of tamoxifen treatment (> 5 years), with early results from the ATLAS (Adjuvant Tamoxifen Longer Against Shorter) and aTTom (adjuvant Tamoxifen – To offer more?) trials indicating small but significant reductions in local recurrence. ATLAS reported local recurrence rates of 21.4% vs. 25.1% and aTTom 16.7% vs. 19.3% in patients with extended tamoxifen treatment compared with those with only 5 years.^{123,124} More mature data will be needed before firm conclusions and guidelines can be agreed. There have been several RCTs evaluating endocrine treatment, duration of therapy and sequencing. The key published trials are summarised in Table 10.

	Year	Patient selection	Number of patients	Treatment	Primary outcome
NATO (Nolvadex Adjuvant Trial Organisation) RCT ¹²⁵	1985	Node – ve pre-menopausal Node +ve/-ve EBC post-menopausal	1285	2 years tamoxifen vs. no treatment	Improved OS in treatment group
NSABP Double-blind RCT ¹²¹	1989	ER +ve EBC node –ve pre and post-menopausal	2644	5 years tamoxifen vs. placebo	PFS increased with tamoxifen 83% vs. 77% ($p < 0.0001$)
NSABP re-randomised Double-blind extension trial ¹²⁶	2001	Continued adjuvant treatment in ER +ve EBC node –ve pre and post-menopausal	1172	Further 5 years tamoxifen vs. placebo	No additional benefit in DFS or relapse-free survival (at 7 years follow-up)
Meta-analysis of ABCSG-8 (Austrian Breast and Colorectal Study Group), ARNO-95 (Arimidex-Nolvadex) and ITA (Italian Tamoxifen Anastrozole) ¹²⁷	2006	Hormone sensitive EBC post-menopausal	4006	Anastrozole or tamoxifen after 2-3 years tamoxifen	Significant improvement in DFS and OS in patients switching to anastrozole
ATAC (Arimidex, Tamoxifen, Alone or in Combination) RCT (10-year follow-up) ¹²⁸	2010	EBC post-menopausal	9366	Anastrozole vs. tamoxifen vs. anastrozole + tamoxifen	Improved DFS, time to recurrence and decreased incidence of contralateral breast cancer for anastrozole vs. tamoxifen ($p = 0.04$, 0.001 and 0.01 respectively) No improvement in OS.
BIG (Breast International Group) 1-98 RCT ¹²⁹	2005	EBC post-menopausal	8010	Letrozole vs. tamoxifen	Improved DFS and OS for letrozole ($p < 0.001$)
IES (Intergroup Exemestane Study) Double-blind RCT ¹³⁰	2004	EBC post-menopausal	4742	5 years tamoxifen vs. 2-3 years tamoxifen + exemestane	Improved DFS and OS for tamoxifen + exemestane
MA-17 ¹³¹	2005	Receptor +ve, post-menopausal women	5187	Previous tamoxifen followed by letrozole vs. placebo	Improved DFS and distant DFS for letrozole patients Improved OS in node +ve patients
TEAM (Tamoxifen Exemestane Adjuvant Multinational) RCT ¹³²	2011	Receptor +ve, post-menopausal women	9779	2-3 years tamoxifen and exemestane vs. 5 years exemestane	No difference in DFS at 5 years

Table 10 Key trials evaluating endocrine treatments, duration of therapy and sequencing RCT, randomised control trial; NSABP, National Surgical Adjuvant Breast and Bowel Project; EBC, early breast cancer; -ve, negative; +ve, positive; PFS, progression free survival; DFS, disease free survival; OS, overall survival.

Aromatase inhibitors (AIs) prevent the conversion of androgens to oestrogens in peripheral tissues, which is most relevant in post-menopausal women in whom this is the main source of oestrogen. The ATAC trial compared anastrozole with tamoxifen and 10-year results showed an absolute rate reduction of 4.3% in breast cancer recurrences and 2.6% reduction in distant metastasis in patients taking anastrozole.¹²⁸ The Breast International Group (BIG) 1-98 trial compared letrozole with tamoxifen, and showed improved DFS in favour of letrozole.¹²⁹ However, only the BIG 1-98 trial showed a significant improvement in OS in patients taking letrozole (85.4% vs. 81.4%) after median follow-up of 8.7 years.¹²⁹ A meta-analysis of trials comparing AIs with tamoxifen in post-menopausal women concluded that AIs resulted in significantly lower recurrence rates, either as monotherapy or after 2-3 years of tamoxifen (AI switch).¹³³

Several trials assessed the benefit of sequential AIs after tamoxifen and results showed superiority over first-line AI therapy with a reduction in relapse-free survival and OS.¹²⁷ The TEAM (Tamoxifen Exemestane Adjuvant Multinational) trial and one arm of the BIG 1-98 trial compared patients receiving five years of AI with those receiving AI switch and demonstrated no difference in DFS.^{132,134} Endocrine therapy is the most important systemic treatment in ER-positive patients and significantly decreases recurrence and breast cancer mortality.^{124,135} Table 11 summarises the recommendations for adjuvant endocrine treatment.

Menopausal status at time of diagnosis	Recommendation
Pre-menopausal	5 years tamoxifen
Post-menopausal	5 years anastrozole or letrozole
Post-menopausal women after 5 years tamoxifen	Consider anastrozole, letrozole or exemestane in high risk patients
Women after 5 years of aromatase inhibitor	No level 1 evidence at present Consider continuing current treatment in high risk patients

Table 11 Recommendations for adjuvant endocrine treatment

1.2.4.3 Biologically-targeted therapy

Trastuzumab is a recombinant humanised monoclonal antibody that inhibits the HER2 receptor by binding to it. It causes decreased tumour proliferation and suppresses angiogenesis and has significantly improved survival in metastatic breast cancer. There were two pivotal trials which demonstrated that trastuzumab was effective as both a single agent in patients with metastatic breast cancer who had previously had chemotherapy and also when used in combination with other chemotherapy agents.^{136,137} Table 12 summarises the main RCTs testing adjuvant trastuzumab in HER2 positive patients. Longer-term follow-up from these studies has confirmed that in patients with tumours overexpressing HER2, trastuzumab consistently decreases local recurrence and improves overall survival by approximately a third.¹³⁸⁻¹⁴² The HERA trial evaluated extended trastuzumab treatment (2 years vs. 1 year) and reported no added benefit from extended treatment.¹⁴³ Lapatinib is a newer therapy, which interrupts HER2 and EGFR pathways. It is used in combination with other chemotherapy agents in patients with advanced or metastatic breast cancer with some promising results.¹⁴⁴

Trial	Number of patients	Treatment regimen	Hazard ratio	
			DFS	OS
National Surgical Adjuvant Breast and Bowel Project B31 ¹⁴⁰	3968	AC-T vs. AC-T and H(concurrently)	0.52	0.61
Intergroup N9831 ¹⁴⁰		AC-T AC-T H vs. AC-T and H(concurrently)		
Breast Cancer International Research Group (BCIRG)-006 ¹³⁸	2147	AC-T	0.64	0.63
	2148	T and P and H (concurrently)	0.75	0.77
Herceptin in Adjuvant Breast Cancer (HERA) ¹⁴¹	3501	Standard adjuvant chemotherapy then H	0.76	0.85 (NS)

Table 12 Adjuvant trastuzumab randomised controlled trials

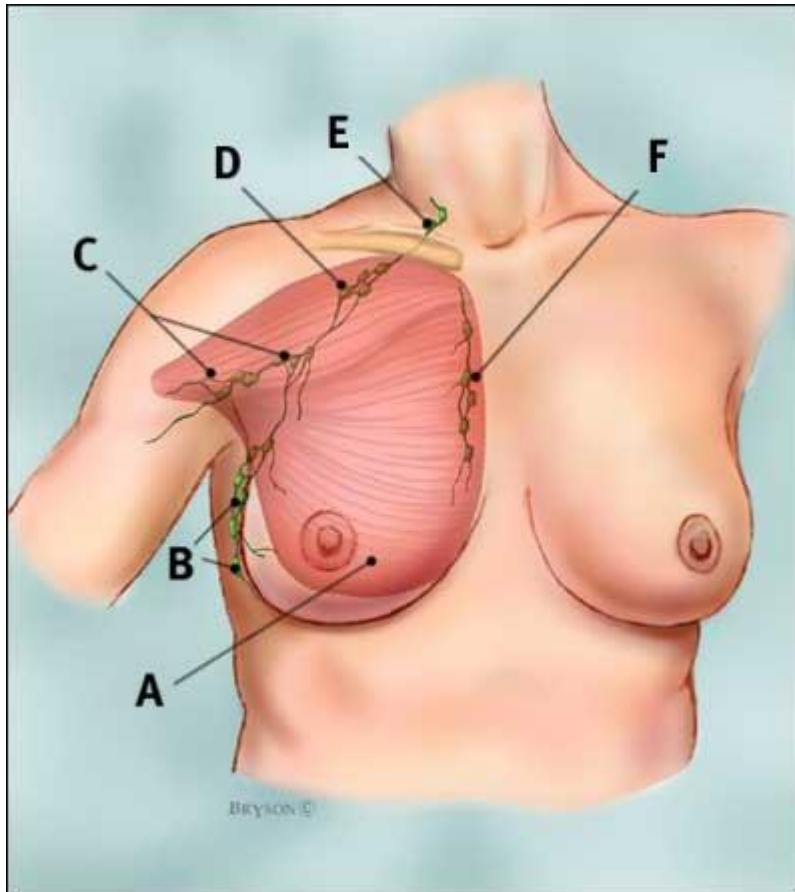
AC, anthracycline; T, taxane; H, trastuzumab (Herceptin); P, carboplatin; DFS, disease-free survival; OS, overall survival; NS, not significant.

1.3 Anatomy of the lymphatic system of the upper limb

The lymphatic system begins in the interstitium in the form of capillary vessels, or initial lymphatics, organised as an anastomosing network or plexus. The initial lymphatics, diameter 80-130 µm, possess a thin wall of endothelial cells supported by an incomplete basement membrane.¹⁴⁵ The outer surface is tethered to the surrounding tissues by anchoring filaments which assist in dilating the vessels, e.g. in oedema.^{146,147} The edges of the endothelial cells overlap to form valves which allow fluid to enter under pressure gradients. The density of the initial lymphatics is highest in the upper dermis of the skin, the density decreasing progressively in the deeper dermis and subcutis. The initial lymphatics eventually join into larger vessels, the pre-collectors and collectors, often running alongside the veins, and passing centrally to the regional lymph nodes. Collector lymphatics possess smooth muscle in their walls and have a thin external connective tissue coat, similar to small veins.¹⁴⁸ In the larger vessels, valves ensure unidirectional flow of lymph. The

superficial lymphatics may pierce the deep fascia and enter the deep lymphatic system. Deep lymph vessels follow the main neurovascular bundles to the lateral axillary nodes (often passing through 2 or 3 cubital nodes at the elbow).^{148,149} The efferent lymphatics leading from the apical group of axillary lymph nodes (see below) drain into the subclavian lymphatic trunk or duct, which joins the bloodstream at the subclavian vein via a lymphaticovenous anastomosis near the junction of the internal jugular vein.

The axillary nodes receive more than 75% of the lymph from the breast. There are between 20-40 axillary nodes, which are divided into five main groups; anterior/pectoral, posterior/subscapular, lateral, central and apical groups (Figure 1). Collectively, these drain the entire upper limb, breast and trunk above the umbilicus. The nodes are described in relation to pectoralis minor in the surgical setting. Those lying below and lateral to the pectoralis minor are called level 1, those posterior to the muscle level 2, and the nodes between the lower border of the clavicle and the upper border of pectoralis minor are level 3 nodes (Figure 2). ALND involves clearance of all the nodes to levels 1, 2 or 3.¹⁴⁸



- | | |
|---|-------------------------|
| A | Pectoralis major muscle |
| B | Level 1 nodes |
| C | Level 2 nodes |
| D | Level 3 nodes |
| E | Supraclavicular nodes |
| F | Internal mammary nodes |

Figure 1 Levels of clearance.

Reproduced by kind permission of Clinically Oriented Anatomy¹⁵⁰

Superficial lymphatic vessels begin in cutaneous plexuses of the hand, and in the forearm run alongside the superficial veins. The superficial lymphatics pierce the deep fascia and enter the lateral axillary nodes or deep lymphatic vessels. Deep lymph vessels follow the main neurovascular bundles to the lateral axillary nodes.^{148,149}

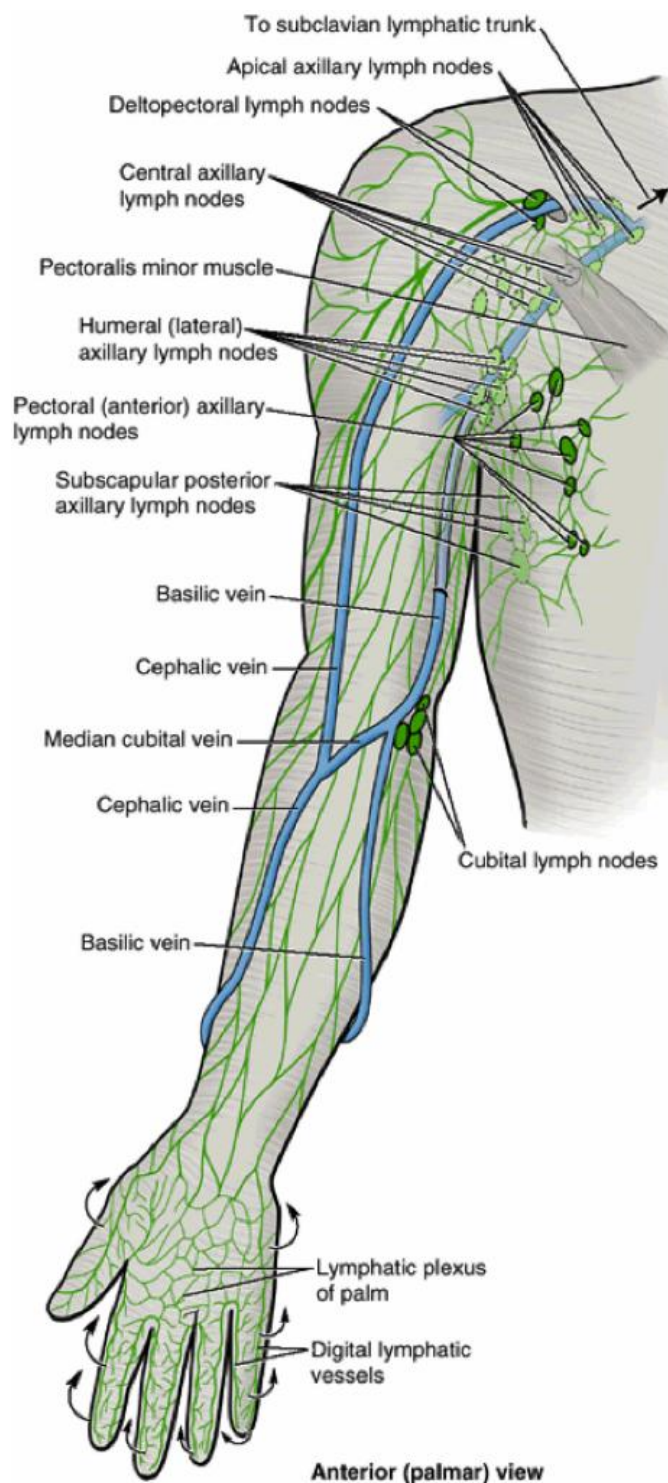


Figure 2 Lymphatic drainage of the upper limb

Reproduced by kind permission of Clinically Oriented Anatomy¹⁵⁰

1.4 Physiology of lymphatics

The lymphatic system has three main functions: preservation of fluid balance, defence and nutritional.¹⁴⁷ Lymph has a daily circulating volume estimated at 2-3 litres.¹⁵¹ The lymphatic system is one of the major routes for absorption of nutrients from the gastrointestinal tract, and is principally responsible for the absorption of digested fats in the form of chylomicra. The defence function acts to carry foreign material such as viruses, bacteria and antigens to the lymph nodes. Here, they are filtered and phagocytosed, potentially stimulating an immune response leading to entry of lymphocytes into the efferent lymph for transport to the bloodstream. For this reason, efferent lymph has a higher white cell count than afferent lymph.¹⁴⁷

The exact filling mechanism of lymphatic fluid entering the initial lymphatics is unclear, but is often likened to that of the filling of a Pasteur pipette. The initial lymphatic plexus is emptied by compression secondary to tissue movement and then re-expands due to the tension in the tethering filaments. This causes the intra-lymphatic pressure to fall below the interstitial fluid pressure and this pressure gradient drives interstitial fluid into the lumen of the lymphatic capillaries. Up to a certain point, the higher the interstitial fluid pressure and volume, the greater the lymph flow. Interstitial pressure and volume are influenced by capillary filtration rate and provide the functional link between capillary filtration and lymph flow; lymph drainage and capillary filtration rate are normally in balance to avoid oedema.¹⁴⁷

The pressure in the venous outlet at the neck is higher than in the lymphatic system, so lymph has to be pumped along the lymphatics. Flow along the initial lymphatics (lacking smooth muscle in their walls) is promoted extrinsically by deformation of tissues by smooth muscle contraction, arterial pulsation or (in the chest and abdomen) respiration and peristalsis. The lymphatic vessels containing abundant smooth muscle show spontaneous contractions of 8-15 cycles per minute, and can pump up to 40-50 mm Hg.¹⁴⁷ The valves exist in all lymph channels to prevent retrograde flow and each segment of lymph vessel between valves acts as a separate pump. These segments are likened to mini-hearts linked in series, maintaining an intrinsic pumping mechanism. The frequency of contraction and stroke volume increases with distension. This allows each lymphatic segment to increase output in response to the increased output from the segment before it. Larger lymphatics are also under sympathetic control, which allows a further increase in the frequency of lymphatic contraction. In haemorrhage, increased lymphatic contraction frequency and contractility allows enhancement of transfer of interstitial fluid into the depleted circulation.^{146 147}

1.4.1 Physiology of lymph production

Lymph is derived from interstitial fluid that flows into lymphatics, and the interstitial fluid derives from the blood plasma. The capillary wall is semi-permeable and fluid containing water, plasma proteins and small molecules such as electrolytes leaks out continuously. This filtration is described by the Starling principle of fluid exchange, which can be stated as:

$$\text{Net filtration rate } (J_v) \propto (\text{net hydrostatic drive} - \text{net osmotic suction})$$

The movement of fluid out of a capillary is thus driven by the balance of the hydrostatic and colloid osmotic pressures on either side of the capillary wall. The proportionality factor represents the surface area and hydraulic conductance of the capillary wall. The traditional view has been that arteriolar end of capillaries filter fluid and the venular end of capillaries re-absorb most of it, thereby preserving tissue volume. This view is no longer supported by modern evidence, which has shown that fluid moves from capillary to interstitial space along the entire length of the vessel in the steady state, falling almost to zero at the venular end but with no re-absorption.¹⁵² There is a slight excess of filtration over absorption, and it is this excess fluid, containing small amounts of protein, that makes up lymph.¹⁴⁷ A shift in the balance of the forces in the Starling equation will result in a shift in the filtration rate. If the net filtration rate increases then lymph flow would have to increase to prevent tissue swelling. A higher filtration rate therefore represents higher lymph production if the tissue volume is constant.¹⁵¹

Approximately 8 litres of fluid is filtered per day and enters the lymphatic system as afferent lymph. Perhaps half of this volume is removed by absorption as it passes through lymph nodes, leaving ~4 litres to re-enter the bloodstream in the neck.¹⁴⁷ The plasma volume is itself only ~3 litres and so the entire plasma volume, excluding plasma protein, leaves the blood stream and recirculates via the lymphatic system every ~9 hours.¹⁵³

Introduction to breast cancer-related lymphoedema

Breast cancer-related lymphoedema (BCRL) presents as a chronic swelling of the arm following axillary lymph node surgery as part of the surgical treatment for breast cancer. It was originally described by Handley in 1908 as the brawny arm of breast-cancer, producing discomfort and misery.¹⁵⁴ In 1921, Halsted described the same condition as a complication of radical mastectomy and coined the phrase 'elephantiasis chirurgica' or 'surgical elephantiasis'.¹⁵⁵ Although dramatic swelling on the scale of 'elephantiasis chirurgica' is now rare, lymphoedema following breast cancer surgery remains a poorly understood and incurable problem.¹⁵⁶

The initial treatment-related trauma to the axilla (either surgery or radiotherapy) is generally agreed to be the catalyst in the development of BCRL, but notably the majority of patients do not develop BCRL. The aetiology of the condition remains incompletely defined and factors other than the primary initiating events are yet to be identified.¹

This thesis aims to further understand the pathophysiology of BCRL. Before considering the pathophysiology and aims of this thesis, a clinical overview of BCRL will be given.

1.5 Definition of BCRL

The morbidity of BCRL spans across physical, functional and psychological domains and as such should be defined with multi-dimensional methodology including subjective and objective findings.¹ Current definitions are largely focused on quantitative between-arm volume differences or circumferential measurements. These methods do not necessarily identify patients with more subtle swelling, which is only noticeable on physical examination. These methods would also exclude the subjective findings by patients.^{1,157} The nature of BCRL also means that it can be patchy, spare certain parts of the upper limb,¹⁵⁸ and may also progress towards fibrosis and muscle atrophy,¹⁵⁹ making the volume measurement inaccurate as a sole diagnostic tool. The lack of a standard definition in the literature and the lack of a standardised and reliable method of quantifying lymphoedema have resulted in the absence of a universally accepted definition of BCRL.¹⁵⁷ Since there is no consensus, it makes it difficult to reliably draw meaningful comparisons between clinical studies.¹⁶⁰

The definition of BCRL used throughout this thesis is the presence of any one of the following:

- Arm volume difference of 10% or more between the pre-operative (baseline) and post-operative arm measurement of the affected side
- Visible swelling on clinical examination of the limb

There is a natural asymmetry in upper limb volume depending on arm dominance with the dominant arm being 3-5% bigger than the non-dominant arm.^{161,162} This will also be taken into account when comparing both upper limbs.¹⁶³

1.6 Clinical features of BCRL

It has been reported that 75% of cases of BCRL occur within the first year after surgery and 90% of cases will present within three years.^{164,165} There have been reports of latent periods of greater than 20 years, indicating a possible substantial delay between initial surgery and the onset or reporting of swelling.^{58,59} The onset of BCRL can be gradual or sudden and patients sometimes report a precipitating factor, such as lifting something heavy with the arm or a graze leading to a minor infection.¹⁵⁶ After an initial rapid expansion (when capillary filtration rate exceeds lymph drainage), the arm volume tends to plateau and then remains in a steady state.¹⁶⁶

In cases of mild BCRL when the upper limb volume increase does not appear significant, examination may reveal decreased visibility of the subcutaneous veins on the dorsum of the hand and forearm and fullness and rounding of the medial elbow and upper arm contours, indicating the thickening of tissues. It is also important to assess the distribution of swelling along the length of the limb, which varies between patients.⁵⁸ Lymphoedema is often described as a brawny and non-pitting oedema, but in the early stages the swelling is often soft and pits easily on pressure. Given time, the skin texture may continue to change, although the rate of

change varies widely. These changes can range from deepening of skin creases to hyperkeratosis and papillomatosis, which leads to the picture of ‘elephantiasis’, at which point the swelling is usually fixed and resistant to conservative measures.¹⁶⁷

The International Society of Lymphology has developed a three stage scale for classification of a lymphoedematous limb¹⁶⁸ (Table 13).

The appearance of oedema may sometimes represent local tumour recurrence within the axilla in 15% of patients, which should be borne in mind when patients present with swelling of the upper limb.^{58,156}

Stage	Description	Characteristics
I	No or minimal fibrosis	Oedema pits on pressure and reduces with limb elevation
II	Substantial fibrosis	Oedema does not pit on pressure, elevation alone rarely reduces swelling
III	Lymphostatic elephantiasis	Absent pitting, trophic skin changes, further deposition of fat and fibrosis, warty overgrowths

Table 13 Clinical classification of lymphoedema (International Society of Lymphology)¹⁶⁸

1.7 Epidemiology of BCRL

The reported prevalence of BCRL in the literature shows wide variation. The population of breast cancer patients examined comprise those who have had surgery ranging from radical mastectomy to breast-conserving surgery, and have

had various levels of axillary lymph node dissection as well as differing regimens of radiotherapy, chemotherapy and endocrine therapy. This by itself may account for part of the variation in the prevalence of BCRL.¹ From earlier studies there are documented rates of BCRL ranging from 6.7 to 62.5% in a review of nine series published between 1908 and 1950.¹⁶⁹ A subsequent series of radical mastectomies from 1940 to 1961 was reviewed by Hughes and Patel, who reported a BCRL rate of 49.2%.¹⁷⁰ The majority of these patients underwent radical mastectomies with or without radiotherapy to the axilla.

As surgical intervention became more conservative, the rates of BCRL were also found to fall, although they were still very variable. More recent data from larger series with a regular follow-up period provide a more accurate prevalence of BCRL. Mortimer *et al* (1996) reported a rate of BCRL of 28% in 1249 patients who had undergone ALND and were followed up for a period of 9.5 years.¹⁷¹ A similar rate of 24% was found by Schunemann *et al* (1998) in a series of 5657 patients who were followed up for 11 years.¹⁷² A lower rate of BCRL was found by Herd-Smith *et al* (2001) in a study of 1278 patients treated from 1989-1997, with a prevalence of 16%¹⁷³ with a more recent prospective single site study by Clark *et al* (2005) reporting a rate of 20.7% after 36 months follow-up.¹⁷⁴ DiSipio *et al* (2013) have conducted a systematic review and meta-analysis of 72 studies with 29,612 women from 2000-2012 and estimated an incidence of 19.9% (range 8.4% - 21.4%) in patients undergoing ALND.¹⁷⁵ These figures have led to the often-quoted 1 in 5 risk of patients developing BCRL following ALND.⁵⁸

1.8 The burden of BCRL

Breast cancer is the most common cancer affecting women worldwide with 1.38 million women diagnosed every year.¹⁷⁶ In all, approximately 40% of patients will be node-positive and may require axillary lymph node clearance. With an estimated 20-25% risk of BCRL, it is clear that this remains a significant clinical problem. BCRL presents as a chronic swelling of the arm, either local or regional, and can be associated with significant physical, functional, psychological and social morbidity.

1,60

1.8.1 Physical morbidity

Patients with BCRL may report symptoms such as sensations of fullness and discomfort in the arm. Other symptoms include skin changes, decreased range of joint movement, pain and recurrent erysipelas or infections.^{1,60,156,177} Disabilities include limb heaviness, reduced movement and impaired function with the increased size and weight of the limb leading to progressive musculoskeletal and joint problems.¹⁷⁸ A rare complication of BCRL is the development of cutaneous malignancy in long-standing lymphoedema such as squamous cell carcinoma, melanoma, Kaposi sarcoma and lymphoma.¹⁷⁸ Stewart-Treves syndrome is a lymphangiosarcoma arising in the presence of chronic lymphoedema with an incidence of 0.03%.¹⁷⁹ The mean survival is 24 months, with a five-year survival rate of approximately 10%.¹⁸⁰⁻¹⁸²

1.8.2 Psychological morbidity

The disfiguring, disabling and chronic nature of BCRL places patients at risk of significant psychological and social sequelae.¹⁸³ In one of the earliest studies to explore psychological morbidity associated with BCRL, patients were found to experience poorer adjustment to their illness and considerable difficulty with regard to home environment and sexual and interpersonal relationships.¹⁸⁴ A study by Woods *et al* (1995) using the Psychosocial Adjustment to Illness Scale (PAIS) questionnaire, showed 86% of patients had a measurable psychosocial maladjustment at the time of referral with lymphoedema.¹⁸⁵ A more recent prospective cohort study of 633 breast cancer patients showed that patients with BCRL had significantly worse emotional well-being and adjustment to life compared with those without BCRL.¹⁸⁶ A review by McWayne *et al* (2005) found higher levels of anxiety, depression, increased frustration and anger, as well as a worse quality of life (QoL).¹⁸³ Patients also experience problems with dress, with some reporting loss of interest in appearance with subsequent loss of self-esteem and avoidance of social activities leading to further social isolation.^{183,184}

1.8.3 Financial implications

Lymphoedema causes significant morbidity and as such there is a financial cost, which has implications for health service providers and workforce planners. The costs include routine care such as follow-up appointments and therapies, but there are additional economic concerns such as patients having to give up paid employment as they are no longer able to perform duties required of them involving the affected limb.^{187,188} In a survey of lymphoedema patients carried out

in the UK by Moffatt *et al* (2003), over 80% of patients had taken time off because of this, with 2% having to change jobs, and a further 8% having to give up work altogether.¹⁸⁹ A study in the United States of America estimated the economic cost of BCRL and found that in a group of 1877 breast cancer patients studied, BCRL patients had significantly higher medical costs compared to non-BCRL patients (\$23,167 vs. \$14,877 respectively). These costs were attributed to imaging, increased outpatient care and multiple clinic visits.¹⁸⁸

1.9 Risk factors for BCRL

The initiating factors of BCRL are accepted to be axillary surgery and radiotherapy, but the pathophysiology remains poorly understood. It was traditionally thought that a 'stopcock hypothesis' explained the mechanism, with damage to the axillary drainage pathways impairing drainage of lymph from the whole arm causing interstitial fluid to build up in the arm.^{58,190} There are many other features of BCRL that do not fit with this traditional view. This includes the fact that the majority of women who undergo axillary lymph node dissection and/or radiotherapy do not develop BCRL, it sometimes develops after many years, and the swelling is often non-uniform suggesting that this hypothesis is too simplistic. It seems likely that many factors influence the risk of developing BCRL.

1.9.1 Surgical intervention

1.9.1.1 Breast surgery

There have been significant changes to breast cancer treatment over the past 125 years with importance given to associated morbidity as well as mortality. Halsted's radical mastectomy resulted in an incidence of BCRL of up to 62.5%.¹⁶⁰ With the introduction of more conservative approaches to the surgical management of breast cancer, the incidence of BCRL has decreased. A recent study has shown there to be no statistically significant difference in BCRL rates between MRM and BCS.¹⁹¹

1.9.1.2 Axillary surgery

Accurate assessment of axillary node status is essential for staging, prognosis and guiding (neo-)adjuvant treatment decisions. ALND has previously been the standard approach for staging the axilla, but this method is associated with significant morbidity, including BCRL. In addition to this, the majority of women with early-stage breast cancer are node negative, and these patients derive no benefit from an ALND.⁷⁶

ALND is associated with significantly more morbidity than SLNB including pain, neurosensory changes, residual shoulder movement and BCRL.^{76,192,193} The rate of BCRL in patients undergoing SLNB has been quoted as low as 4-6%.^{194,195} Two randomised control trials have shown a significant reduction in the subjective rate of arm swelling in patients undergoing SLNB compared to those having ALND.^{79,196} The ALMANAC trial was a multicentre randomised study comparing SLNB with

standard axillary treatment, *i.e.* level 1-3 ALND or 4NAS. The results showed that patients undergoing standard axillary treatment reported moderate to severe BCRL more often than SLNB patients at 12 months post-surgery (subjective reporting of 5% vs. 13%, $p < 0.01$). However, objective measures at 12 months post-surgery did not show a statistically significant difference between the two groups.⁷⁶ The odds ratio for the development of BCRL in patients undergoing SLNB compared with ALND in the main prospective RCTs is summarised in Table 14, and shows significantly less BCRL in patients undergoing SLNB.

	Recruitment years	Number of patients	Odds ratio (95% confidence interval) for BCRL
Sentinella/GIVOM ⁷⁷	1999 – 2004	697	0.48 (0.3 – 0.8)
Purushotham <i>et al</i> ⁷⁹	1999 – 2003	298	0.30 (0.18 – 0.68)
NSABP B32 ¹⁹⁷	1999 – 2004	3983	0.52 (0.43 – 0.65)
ALMANAC ⁷⁶	1999 – 2003	1031	0.37 (0.23 – 0.60)
Z0011 ¹⁹⁸	1999 - 2004	891	0.52 (0.26 – 1.06)

Table 14 Morbidity of sentinel lymph node biopsy vs. axillary lymph node dissection in key prospective randomised trials

NSABP, National Surgical Adjuvant Breast and Bowel Project; ALMANAC, Axillary Lymphatic Mapping against Nodal Axillary Clearance, Z0011, The American College of Surgeons Oncology Group (ACOSOG) Z0011 trial

4NAS has been shown to produce lower rates of BCRL compared with ALND, with one recent study showing BCRL rates of 2.2% and 12.3% respectively.¹⁹⁹

1.9.2 Radiotherapy

The pathophysiology of BCRL in patients undergoing radiotherapy is thought to be complex. It may include a radiotherapy-induced fibrosis, causing venous and lymphatic vessel obstruction and lymphocyte depletion or fatty replacement

following lymphocyte depletion leading to focal fibrosis.^{200,201} Whilst studies performed *in vitro* and *in vivo* in human and animal studies appear to show that lymphatic vessels are relatively insensitive to radiation, radiotherapy causes development of fibrosis of surrounding structures and delays the normal growth of lymphatics within tissues.²⁰² Lymph nodes, however, have been found to be radiosensitive with radiation decreasing their filter function and altering their immune function.²⁰³ With this in mind, it is thought that early lymphoedema may be due to impairment of normal lymphatic regeneration, and late lymphoedema due to delayed soft tissue fibrosis.²⁰¹

The variables potentially contributing to the development of BCRL are the use of X-ray irradiation vs. megavoltage irradiation, the dose of irradiation and the treatment field.¹⁷² Conventional X-ray radiotherapy was initially used after surgery with high rates of BCRL, but the change to megavoltage irradiation significantly decreased the incidence of BCRL.¹⁷² There is a marked relationship between dose of radiotherapy and incidence of morbidity. Small changes in the percentage of dose administered can lead to significant increases in morbidity.²⁰⁰ The field of irradiation also affects the incidence and severity of BCRL. Historically, the field would include the chest wall, axilla, supraclavicular fossa and internal mammary lymph nodes. Radiotherapy to the axilla considerably increases the incidence of BCRL,^{60,173,204,205} with reports of an incidence of BCRL of 38.3% in patients undergoing ALND and radiotherapy.¹⁶⁰

The AMAROS trial measured the rates of lymphoedema at 1, 3, and 5 years in patients with positive SLNs undergoing either cALND or axillary radiotherapy.¹⁰² The final analysis of the trial reported a 1-year rate of lymphoedema of 40% in the group undergoing ALND compared with 21.7% in the group of patients treated with axillary radiotherapy. This statistically significant difference was also seen at 3 years (29.8 vs. 16.7%) and 5 years (28.0 vs. 13.6%) respectively. At 5 years, the axillary recurrence rate was 0.43% for patients undergoing cALND and 1.19% in the axillary radiotherapy group. This suggests that radiotherapy can offer similar results to ALND, but with an accompanying significant reduction in rates of BCRL.¹⁰²

1.9.3 Chemotherapy

Several studies have reported an association between BCRL and patients undergoing radiotherapy and adjuvant chemotherapy.^{200,204,206} Norman *et al* (2010) conducted a study of 631 patients, and found an increased hazard ratio (HR) in patients undergoing ALND compared with SLNB (HR 2.61, 95% confidence interval (CI) 1.77 – 3.84) and patients receiving anthracycline-based chemotherapy had a HR of 1.46 (95% CI 1.04 – 2.04) compared to those who did not receive chemotherapy. On multivariate analysis, the combination of ALND and chemotherapy increased the hazards ratio 4-5 fold for BCRL.¹⁶⁵ Fontaine *et al* (2011) were the first to publish a prospective analysis of BCRL in early breast cancer patients undergoing concomitant post-operative radiotherapy and anthracycline-based chemotherapy +/- taxanes. The incidence of BCRL was 44% in the group receiving taxanes, three times higher than the non-taxane group, although a complete resolution of BCRL was seen in 13% of patients in the taxane group.²⁰⁷ Studies into the mechanism of

the development of oedema in patients receiving taxanes have been conducted with capillaroscopy and capillary filtration tests using ^{99m}Tc -albumin. These have concluded that there is an abnormality in the capillary permeability and also a progressive accumulation of proteins in the interstitial space.²⁰⁸ It has also been suggested that the axillary radiotherapy administered to this group, in addition to treatment with anthracycline- and taxane-based chemotherapy, contributed to axillary fibrosis and the subsequent development of BCRL.²⁰⁷

1.9.4 Nodal status

Several retrospective studies have suggested that lymph node positivity is related to the development of BCRL,^{160,173,174,209,210} however, all these patients had axillary radiotherapy administered if they were found to be node positive, which by itself would affect the incidence of BCRL. The total number of nodes removed rather than the specific surgical procedure has been found to have a greater correlation with BCRL.²¹⁰⁻²¹³ Meeske *et al* (2009) interviewed patients 18 months after treatment for breast cancer and observed that if >10 lymph nodes were removed, patients had a 2.6 fold increase in the risk of developing BCRL.²¹² A similar finding was observed by Larson *et al* (1986) where the risk of BCRL was 28% in patients in whom > 10 nodes were removed compared with 9% in patients in whom 1-10 nodes were removed.²¹⁰ A more recent prospective study by Kwan *et al* (2010) studied 997 patients and found that patients with BCRL had more positive lymph nodes compared with those without BCRL (3.3 vs. 0.8).²⁰⁶ However, a further study questioned this relationship. The association between nodal positivity and the development of BCRL was examined in a recent analysis of two prospective studies

of 212 patients undergoing ALND. It was observed that positive nodal status was inversely related to upper limb volume in all patients after correcting for changes in the contralateral arm, raising the possibility that the inverse relationship may be due to node positive patients developing collateral lymphatic drainage prior to undergoing ALND.²¹⁴

1.9.5 Infection

Infection has been identified as a risk factor for BCRL in various studies.^{214,215} A prospective study by Petrek *et al* (2001) identified self-reporting history of arm infection or arm injury as being significantly associated with late onset BCRL (> 3 years after diagnosis) but the potential for recall bias limited its validity.²¹⁵ Although venepuncture is a potential source of infection, the association of increased risk of developing BCRL is largely anecdotal.¹⁷⁴ There is no clear evidence of increased risk, but caution should be exercised using the limb at risk of developing BCRL, unless there are overriding clinical reasons.

1.9.6 Patient factors

There is conflicting evidence regarding age being a risk factor in the development of BCRL. Yen *et al* (2009) performed a population-based cohort study of elderly breast cancer survivors (aged 65-89 years) using self-reporting methods, with 14% reporting BCRL after four years follow-up. No association was found between increasing age and BCRL, but the presence of axillary metastases, number of nodes removed and more advanced tumour stage conferred an increased risk.²¹³ A similar study using self-reporting by Meeske *et al* followed 494 patients for 4 years,

specifically looking at age, ethnicity and BMI. They found younger age (<55 years) and elevated BMI (>30 kg/m²) to be risk factors. African-American women were more likely to develop BCRL compared with white women in this study, but when adjusting for other variables no difference in prevalence was observed.²¹² Kwan *et al*, however, found there was a differential risk of BCRL according to race and ethnicity. It was observed that African-American women, Asian-Americans and Hispanics had an increased risk when compared with white women, but this was not found to be statistically significant.²⁰⁶ Beaulac *et al* assessed upper limb volume measurements in patients following ALND and found that patients who developed BCRL were more likely to be non-white (African-American, Hispanic, Asian and Middle-Eastern), and had decreased QoL scores.²¹⁶ Numerous studies have found a correlation between obesity and BCRL.^{174,206,217,218} A recent meta-analysis found strong levels of support for the relationship between BCRL and BMI with 50% of BCRL patients being overweight or obese.¹⁷⁵ Other studies have suggested risk factors for BCRL including higher socio-economic status,^{206,211} tumour affecting the non-dominant side,^{174,211} menopausal status,²⁰⁹ tumour stage¹⁶⁰ and tumour size,^{173,213} although the level of evidence has been found to be weak.¹⁷⁵

1.9.7 Genetics

It has been suggested that there is a possible inherited genetic susceptibility, which contributes to the pathophysiology of secondary lymphoedema such as BCRL.^{175,219} Despite identification of the risk factors above, there has been relatively little work into the possibility of genetic predisposition. Vascular endothelial growth factor(s) (VEGF) are important in the regulation of lymphangiogenesis and

stimulate cellular responses by binding to tyrosine kinase receptors (VEGFR).²²⁰ Mutations of the VEGFR-3 gene have been identified as a major cause of Milroy disease (primary congenital hereditary lymphoedema),²²¹ mutations with SOX18 linked to hypotrichosis-lymphoedema-telangiectasia syndrome²²² and FOXC2 mutations have been linked to lymphoedema-distichiasis.²²³⁻²²⁵ Newman *et al* (2012) hypothesised that these genes, amongst others, known to be involved in lymphangiogenesis may also predispose to BCRL. They studied 10 genes in a case-control study of 120 women who had breast cancer surgery. Blood was taken and genomic DNA was extracted and prepared for genotype analysis. They identified genetic loci from VEGFR2, VEGFR3 and RAR-related orphan receptor C (RORC) genes as being statistically significantly associated with BCRL ($p < 0.05$).²¹⁹ The possibility of these genes conferring a predisposition to BCRL lends to potential future work in this area, which may lead to the identification of a 'molecular signature' that could help predict for BCRL.²¹⁹ If a cohort of genetically susceptible patients is identified it might be possible to manage these patients differently, by minimising surgery to the axilla or using some of the new surgical techniques currently being trialled to prevent BCRL (see section 1.12).

1.10 Assessment of BCRL

The diagnosis and severity of lymphoedema is assessed on the basis of limb volume, shape, skin condition and overall function. Among the quantitative measurements of BCRL, the most widespread is assessment of size, based on either circumference or direct measurement of upper limb volume. Other methods for measuring BCRL

also exist but are generally only used in the research setting. These include measurements of lymph flow, tonometry and bioimpedance.

1.10.1 Computer tomography (CT) and magnetic resonance imaging (MRI)

CT can be used to demonstrate the cross-sectional area of the limb and assess the different limb compartments (skin, subcutaneous and muscle). BCRL has been shown to produce markedly increased volume in the subcutaneous compartments with thickening of the skin, but the muscle is relatively unaffected. A 'honeycomb' pattern is also noted, which is due to fibrosis in the subcutaneous tissues.^{161,167,226}

MRI findings are similar to those of CT, but offer greater detail of lymphatic architecture.^{182,226}

1.10.2 Ultrasound (US)

Ultrasound findings in BCRL are those of increased skin and subcutaneous tissue thickness and the absence of echogenic bands beneath the subcutaneous tissues. Although US has not been much used in lymphoedema, in theory future use could include monitoring the results of treatment.^{167,226}

1.10.3 Water displacement volumetry

This is one of the earliest recorded methods for volume measurement and is sometimes thought of as the 'gold standard' with good reproducibility of results. However, this method is time-consuming and messy, which limits its routine clinical use. It is also contraindicated in patients with open skin lesions and does not provide data about localisation of the oedema and shape of the extremity.²²⁷ As a tool to assess BCRL, this is now infrequently used.

1.10.4 Circumference measurement

Arm circumferences can be assessed by tape measurements at certain fixed points along the limb, *e.g.* 10cm proximal and distal to the olecranon process, and comparison made between the ipsilateral and contralateral limb. This method does not take into account the patchy distribution that can be seen in BCRL and is inadequate to accurately quantify BCRL. However, a tape measure can be used to take circumference measurements at 4cm intervals, with a calculation of volume based on the formula for a frustrum of a cone (i.e. a truncated cone).

$$V_{\text{limb}} = \Sigma(X^2 + Y^2 + XY) / 3\pi$$

With X being the circumference at one point on the limb (usually starting at the styloid process on the wrist) and Y is the circumference at a point 4cm up the limb from X. With good technique, the tape measure method has been found to be a reliable method to measure limb volume.²²⁶

1.10.5 Optoelectric volumetry

The Perometer (350S) is a device, which uses infrared light emitting diodes (LEDs). The limb is placed inside a measuring frame, which contains LEDs on two adjacent sides and rows. The limb casts shadows in two planes and on moving the frame along the length of the limb, dimensions along the X and Y axis are measured every 3mm and its volume calculated by a computer. The shape of the limb is also recorded and displayed graphically, and can be used to measure the volume of any part of the limb.^{161,226} The Perometer has been comprehensively evaluated and is increasingly becoming the gold standard.^{228 157,226}

1.10.6 Tonometry

Objective assessment of the depth of soft tissue pitting has been described using a tonometer. This device applies even pressure to the tissues and the depression is recorded in millimetres and serial measurements over time can quantify pitting characteristics. In its current form, it is unsuited to clinical use due to its time-consuming nature and limited clinical application.¹⁶¹

1.10.7 Bioimpedance

Bioimpedance techniques are used in body composition analysis. The impedance spectrum to a small AC current passed through the limb is measured and total water and extracellular water calculations are made.²²⁶ This allows measurement of differences in oedema volume, compared to limb volume measurement, which does not take into account the changes in compartment composition. This has been shown to be reliable and reproducible and can demonstrate subclinical lymphoedema before the development of measurable BCRL.²²⁹⁻²³¹ It has also been found to have a high correlation with perometer readings and may be a cheaper and more practical alternative to perometry.^{231,232} A NIHR-funded multicentre study is currently recruiting breast cancer patients and assessing the concordance between perometer arm measurements and bioimpedance. In addition, the study is assessing if bioimpedance can identify patients at the early stages of developing BCRL before perometry indicates a significant increase in volume, potentially leading to early treatment and improved outcomes in patients with BCRL.

1.11 Management of BCRL

BCRL is a prevalent and usually irreversible side effect of breast cancer treatment, and can lead to progressive swelling and fibrosis requiring lifelong management. The extent of treatment varies greatly in different centres, which reflects the lack of proven efficacy of any one method and the absence of a 'gold standard' for management of this condition.

Patients undergoing axillary lymph node surgery are given standard precautions for management of the ipsilateral upper limb post-operatively. They are advised to pay special attention to skin care and hygiene, avoidance of injections and wounds to the arm, with thorough antisepsis if such an event were to occur. Patients are also advised to avoid rigorous isometric muscle use *e.g.* carrying shopping. Although infection has been identified as a possible risk factor, there is very little evidence to support much of the other information given. It is largely based on anecdotal reports by patients who have found certain events may have precipitated the development of swelling.

Once a diagnosis of BCRL has been made, the treatment strategies can be divided into three main groups: conservative, pharmacological and surgical.

1.11.1 Conservative

Several reviews have attempted to assess the effectiveness of conservative interventions which include compression therapy, manual and lymphatic drainage and medical therapies.^{60,233-235} Recent systematic reviews by McNeely *et al* (2011)

and Oremus *et al* (2012) have updated evidence from RCTs concerning the benefit of conservative treatment for all cancer-related lymphoedema, citing extensive inter-study heterogeneity precluding the assessment of whether any one treatment method is superior to the others.^{236,237}

1.11.1.1 Complex decongestive therapy

Complex decongestive therapy (CDT) is one of the most common forms of treatment consisting of many components, including manual lymph drainage (MLD), multi-layer compression bandaging, therapeutic exercise, self-management education, skin care and elastic compression.²³⁸ CDT involves a two-stage treatment with the first stage administered over a 4-week period by well-trained therapists in an outpatient setting. It consists of skin care and MLD in combination with a range of exercises and a form of compression (typically multi-layered bandaging). MLD uses light massage strokes to first stimulate lymphatic vessels in the trunk and contralateral arm, followed by proximal to distal massage of the affected arm. This aims to stimulate contractility of the lymphatic system and break up fibrotic tissue. The second stage aims to conserve and optimise the results from the first stage and consists of compression garments skin care and continued 'remedial' exercise with light massage as needed. The second stage is largely patient-led at home.^{60,156,168} The wearing of a compression garment has been shown to be significantly better when used in conjunction with exercise and self-massage compared to exercise and self-massage alone.²³⁹

1.11.1.2 Exercise

There is controversy over the role of exercise in breast cancer patients who either have BCRL or are at risk of developing it. The Physical Activity and Lymphoedema (PAL) trial is the largest RCT to date evaluating the effect of weight lifting in patients with BCRL.²⁴⁰ Results suggested that although exercise was found to neither exacerbate nor improve arm volume, significant benefit was found in improvement of pain/tenderness and reduction in the number of lymphoedema exacerbations, which suggests patients can follow exercise programs without fear of worsening BCRL.²⁴⁰ Studies have also used active resistive exercises with weights demonstrating no worsening of BCRL.²⁴⁰⁻²⁴² Weight loss has also been found to result in a significant reduction in upper limb volume,²⁴³ further supporting exercise and weight loss as strategies to improve BCRL symptoms. A follow-up study from the Physical activity and lymphoedema PAL trial assessed the impact of the weight lifting program compared with no exercise in patients at risk of BCRL following axillary lymph node surgery and found that progressive weight lifting did not increase the risk of BCRL.²⁴² Although these two studies provide the strongest evidence with regard to resistance exercises, other RCTs support their findings.²⁴⁴⁻²⁴⁷ The American Lymphoedema Framework Project conducted a systematic review and the evidence for combining resistance and aerobic exercise concluded that this appeared safe, but recommended that larger and more rigorous studies are needed.²⁴⁸ A recent update from the National Lymphedema Framework has recommended the use of aerobic and resistance exercises for patients with and at risk of BCRL. They advise slow and gradual progression, avoidance of intensity and

repetitive overuse and involvement of professionals to tailor exercise programmes.²⁴⁹ The Cancer and Leukaemia Group B (CALGB) is currently recruiting into a RCT for the prevention of BCRL in patients undergoing ALND. The CALGB 70305 trial is testing whether an intervention focusing on improving upper limb function by providing education about BCRL and combining this with light arm weight exercises and using a light compression sleeve during vigorous exercise reduces the incidence of BCRL and improves QoL.²⁵⁰

1.11.1.3 Low level laser therapy (LLLT)

The use of this method in BCRL was first reported in 1995 after studies suggested it could have a stimulatory effect on local fluid circulation and lymphatic vessels, stimulate lymphangiogenesis and stimulate macrophages and the immune system.²⁵¹ Despite methodological flaws and lack of uniformity in studies assessing the efficacy of LLLT, a systematic review by Omar *et al* (2012) concluded that there was moderate to strong evidence for its use in BCRL.²⁵² Ridner *et al* conducted a RCT randomising 46 patients into three groups of MLD, LLLT or MLD followed by LLLT. Clinical and statistical improvement was reported in upper limb volume in all patients, but there was no difference between the groups. They concluded that LLLT may be an alternative option to conventional MLD, but acknowledged that the study was underpowered.²⁵³ Although follow-up was only limited to 30 months, the methodology was robust and the conclusions therefore warrant further study.

1.11.1.4 Hyperbaric oxygen therapy

A non-randomised trial of hyperbaric oxygen (HBO) therapy in 21 breast cancer patients with BCRL and fibrosis after axillary or supraclavicular radiotherapy showed a statistically significant reduction in arm volume at 12 months follow-up. Some patients also reported an improvement in shoulder mobility and soft tissue symptoms.²⁵⁴ A phase II trial by the same group randomised 58 breast cancer patients to receive either HBO or best standard care for lymphoedema. Results showed no beneficial effect of HBO, with no significant difference in arm volumes, functional outcome or QoL between the two groups.²⁵⁵

Various other treatments have been described in the literature, such as pneumatic compression treatment and deep oscillation devices, and although some studies have observed some improvements in symptoms, there have been mixed results between studies using these methods.²³⁷ A Cochrane review of the literature concluded that there was a lack of well-designed, randomised trials in the range of physical therapies and therefore all results should be viewed with caution.²⁵⁶

1.11.2 **Pharmacological**

Pharmacological treatments for BCRL include benzopyrones, diuretics, antibiotics and antioxidants selenium and vitamin E.

Benzopyrones are a group of drugs based on coumarin and have the potential to stimulate proteolysis by tissue macrophages and stimulate lymphatic collectors. A randomised, double-blind, placebo-controlled trial of benzopyrones was studied in

BCRL, and a significant improvement in swelling was reported.²⁵⁷ Another study found no difference in arm volumes over a period of 12 months when treated with benzopyrones for 6 months.²⁵⁸ A Cochrane review (2004) was unable to draw conclusions about the effectiveness of benzopyrones in reducing volume, pain or discomfort in lymphoedematous limbs due to the poor quality of the trials that had evaluated their role.²⁵⁹ Furthermore, benzopyrones are not licensed for use in BCRL in the UK and have been linked to liver toxicity.

Diuretics act to limit capillary filtration by reducing circulating blood volume. This increases protein concentration in the interstitium and can actually lead to increased fibrosis,^{156,182} which is why diuretics are not recommended in the management of BCRL.

A recent Cochrane review (2009) has found inconclusive evidence for the effectiveness of selenium in preventing infective/inflammatory episodes in lymphoedema due to the paucity of properly conducted RCTs.²⁶⁰ A double-blind placebo-controlled randomised trial of vitamin E and pentoxifylline failed to demonstrate any benefit in patients with BCRL.²⁶¹ Antibiotics should only be used in *bona fide* superimposed cellulitis/lymphangitis. Mild skin erythema without systemic symptoms and signs does not necessarily indicate bacterial infection.¹⁶⁸

1.11.3 Surgical

Surgery is generally only considered in cases resistant to conservative measures. Treatments are broadly divided into three main approaches: excisional procedures, lymphatic reconstruction and tissue transfer.

Charles first described the resection or debulking approach in 1912 for lymphoedema of the scrotum,²⁶² and variations on this radical excision of the subcutaneous tissue and skin grafting are still used today. These procedures act to remove redundant skin and subcutaneous tissues rather than address underlying problems with the lymphatics.¹⁸² Although debulking operations are the simplest approach to reduce the size of the limb, they result in extensive scars and morbidity including ulceration, cellulitis, keloid and lymphatic fistulae.²⁶³ More recent techniques for excisional treatment of BCRL have been the removal of subcutaneous fatty tissue through circumferential liposuction. Results from the largest published case series of 104 patients followed up for 15 years have shown that this is an effective method of treatment in patients with non-pitting BCRL who have not responded to conservative treatment.²⁶⁴ However, long-term management does require patients to continue to wear compression garments 24 hours a day to maintain results.^{168,264}

Lymphatic reconstruction involves using microsurgical techniques to bypass lymphatic obstruction and various methods have been attempted since the 1960s.²³⁸ These include the creation of anastomoses between lymphatic vessels

and veins, lymph nodes and veins and proximal and distal lymphatics.¹⁸² Initially anastomoses were done between lymphatic vessels and larger superficial veins such as the saphenous vein, with improvement reported in 44-78% of patients.²⁶⁵⁻
²⁶⁷ Campisi and Boccardo have shown good long-term results in patients undergoing lymphaticovenous anastomoses (LVA), reporting limb volume reduction of 69% and an 87% reduction in the incidence of cellulitis.²⁶⁸ However, the presence of venous hypertension with subsequent lymphatic outflow obstruction led to some high failure rates²⁶⁹ and a move towards using smaller subdermal vessels (0.3-1mm diameter) has emerged.²⁶⁵ Koshima *et al* compared bandaging with lymphaticovenous surgery to bandaging alone and found a reduction in volume of the lymphoedematous tissue of 47.3% and 11.7% respectively.²⁷⁰

Tissue transfer surgery includes autologous lymph node transplantation (ALNT), bone marrow stromal cell transplantation and also lymphatic anastomoses. Lymphatic anastomoses use free muscle flap, greater omentum or dermis flap transfers in an attempt to divert lymphatic drainage via the deep lymphatics.^{238,271} There have been studies combining ALNT with VEGF therapy with results indicating an improvement in lymph node transplantation rates.^{220,224,272,273} Vignes *et al* (2012) challenged the benefits of ALNT and conducted a prospective study of the complications of this technique. They found severe complications existed, such as iatrogenic donor site lymphoedema, which may be partly due to the genetic predisposition putting these patients at risk as discussed earlier in this

chapter.^{219,274} As a result of this, ALNT remains experimental and has not been widely adopted.²⁷⁵

There is no consensus on the timing of surgery in relation to the onset of BCRL, or the type of patients who would benefit from surgical intervention. Very few studies are prospective or controlled, making surgical efficacy difficult to ascertain.²⁷⁵ A recent systematic review of the surgical treatment of lymphoedema found that most reports were based on small numbers of patients with inconsistent measurement techniques, procedure complications were rarely reported and long-term follow-up was lacking.²³⁸ The authors concluded that without a clear benefit from the different types of surgery for lymphoedema, other conventional conservative therapies such as CDT should be utilised and considered the standard of care.²³⁸

1.12 Prevention of BCRL

BCRL remains an incurable condition hence prevention is the ultimate goal. If it could be possible to identify or predict patients who are at risk of developing BCRL and intervene in a way to prevent it, then this would help reduce the morbidity of this condition and the social and economic costs associated with BCRL.

Axillary reverse mapping (ARM) is a technique which attempts to map the drainage of the upper limb using blue dye and preserving these lymphatics at the axilla if it is oncologically safe to do so, since these lymphatics are not thought to be involved in the drainage of the breast.²⁷⁶ Bennett Britton *et al* investigated the drainage

pattern similarity of the breast and upper limb using dual radioisotope imaging (^{99m}Tc and $^{111}\text{Indium}$) in 15 breast cancer patients. Following periareolar and intradermal hand webspace injection prior to ALND, 13/15 patients yielded nodes that had high activity for either ^{99m}Tc or $^{111}\text{Indium}$, implying that the SLNs for the breast and upper limb were different. However, in 2/15 patients, the retrieved nodes showed high activity for both isotopes, implying a convergence of the two drainage pathways with shared SLNs. It was suggested that these patients might have an increased risk of developing BCRL.²⁷⁷ Thompson *et al* first described the ARM procedure, which involves the intradermal or subcutaneous injection of blue dye into the webspace of the hand or upper inner arm at the time of surgery to identify the upper limb lymphatics. In patients undergoing SLNB, radioisotope was used for identification of the SLN as the blue dye was used for upper limb mapping. Initially a prospective non-randomised study of 40 patients undergoing SLNB or ALND was conducted and a significant variation in drainage of upper limb lymphatics was observed. Blue lymphatics and/or blue nodes were identified in 11/18 patients undergoing ALND. In the cases where they were able to identify and preserve these lymphatics, none of the patients went on to develop BCRL.²⁷⁶ A subsequent larger study of 220 patients by this group found ARM lymphatics to be in or near the surgical field of SLNB in 40.6% of patients. It was speculated that if these lymphatics had not been identified, they would have been at significant risk of disruption during axillary surgery and subsequent progression to BCRL. A small crossover rate (2.8%) i.e. nodes that were positive for ARM and SLN was observed, indicating common drainage channels of the upper limb and breast. There was no

evidence of BCRL at 6 months in patients in whom the ARM draining nodes were preserved.²⁷⁸ A phase II trial recruited 156 patients in whom all patients initially had SLNB with 42 patients undergoing completion ALND. ARM lymphatics and nodes were identified in all patients and preserved in 144/156 patients. BCRL rates of 3.5% and 7% were found in SNLB and ALND patients respectively (mean follow-up 9.4 months), with rates of 2.9% vs. 18.8% if patients had their arm lymphatics preserved or transected.²⁷⁹ The follow-up in both these studies is too short to form robust conclusions, but the preliminary results appear promising.

Boccardo *et al* have used ARM in combination with lymphaticovenous anastomoses in a procedure called LYMPHA (lymphoedema microsurgical preventative healing approach). This involves performing LVA between arm lymphatics and collateral branches of the axillary vein at the same time as ALND to prevent BCRL.²⁸⁰ A prospective randomised study by this group compared patients undergoing ALND with LYMPHA and a control group of ALND without LYMPHA, with 23 patients in each group. After 18 months follow-up, the LYMPHA group had a rate of BCRL of 4.3% compared with the control of 30.4%.²⁸¹

There are a number of problems still to be resolved with the ARM procedure. One issue has been the identification rate of ARM nodes using blue dye alone, but a more recent fluorescence imaging system appears to have improved this.²⁸² This method utilises injection of water-soluble indocyanine green as a contrast agent, which can be detected by near-infrared imaging systems.²⁸³ A more important

issue is whether it is safe to assume ARM nodes will not be involved in metastasis of the primary breast cancer as anatomically there are lymphatic interconnections between the drainage of the upper arm and breast.²⁸⁴

The ARM procedure and its combination with LYMPHA show some promising results. However, there is still much work that needs to be done and long-term follow-up studies are required before concluding that these methods are effective and oncologically safe with regard to preventing BCRL.

1.13 Pathophysiology and pathogenesis of BCRL

The traditional view of the pathophysiology of BCRL (the stopcock hypothesis) is that removal of the axillary nodes reduces lymph flow from the whole arm, resulting in the accumulation of protein-rich oedema fluid in the interstitium.¹⁷⁸

However, there are many features of BCRL that are difficult to explain. The majority of patients undergoing axillary lymph node clearance (approximately 75%) do *not* develop BCRL, despite undergoing similar surgery to those who do. In patients undergoing the less invasive SLNB, with only 1-2 axillary nodes removed, 5-6% will still develop BCRL. The latent period is also variable, with the post-operative swelling never resolving in some patients, many developing BCRL months or years after the surgery, and some even developing BCRL 20 years after their breast cancer treatment. The swelling is often non-uniform, sparing some parts of the upper limb whilst other areas are grossly abnormal. It might be expected that as all parts of the arm drain through the same lymph nodes, so all parts should swell. One study has

shown that protein concentration of the interstitial fluid in the ipsilateral swollen arm is in fact *lower* than in the contralateral arm, not higher, and that the decrease in concentration is inversely proportional to the degree of swelling.²⁸⁵ A further conundrum is the apparent abnormalities reported in the *contralateral* arm of women with BCRL. A study imaging the upper limbs of patients with BCRL found that lymphatic vessels were wider in the contralateral arm of BCRL patients compared with the contralateral arm of women treated for breast cancer but without BCRL.¹⁴⁵ The contralateral arm abnormalities add weight to a constitutional predisposition hypothesis.

Research into interstitial fluid characteristics, lymphatic clearance studies and lymphovenous communications have all contributed to providing more knowledge regarding the pathophysiology of BCRL.

1.13.1 Lymphatic clearance studies

Lymph flow from a tissue is difficult to measure directly in humans, because the flow is very slow, the vessels fragile and the fluid colourless.⁵⁸ However, radioisotopes can be used to measure the lymphatic clearance rates. This technique involves the injection of a radiolabelled macromolecule into the dermis, subcutis or muscle and measuring the lymphatic removal rate constant (k) for the macromolecule using scintillation detectors (providing radioactive counts but no images) or a gamma camera (providing anatomical images and counts) in the method known as quantitative lymphoscintigraphy.^{58,286}

The choice of radiolabelled macromolecule varies, and much research has been done using technetium-99m-human immunoglobulin G ($^{99m}\text{Tc-HIG}$). $^{99m}\text{Tc-HIG}$ has been measured in the forearm epifascial compartment (subcutis and skin), forearm subfascial compartment (skeletal muscle) and hand in breast cancer patients with and without BCRL. Lymph flow from the oedematous forearm subcutis was found to be moderately impaired compared to the contralateral side, but certainly not stagnant, contrary to the concept of lymphostasis. A comparison of the subfascial and epifascial compartments showed that the local lymph flow was 2-3 times faster in forearm muscle than the subcutis, indicating a much faster fluid turnover in the muscle compartment. This may reflect the higher density of blood capillaries in muscle than subcutis, which would generate more capillary filtrate and hence more lymph per unit time.²⁸⁷ The hand is sometimes spared when the rest of the arm is swollen. A study of lymph flows from the hands in two groups of women, one with hand swelling and one with spared hands, yielded unexpected results. Lymph flow was *faster* in the contralateral hand of the swollen hand group when compared with the ipsilateral swollen hand, or with either hand of the spared hand group.²⁸⁸ This added weight to the possibility of a contralateral arm abnormality in some women and a constitutive predisposition to BCRL.⁵⁸

1.13.2 Interstitial fluid characteristics

As previously explained, if BCRL resulted purely from a disturbance in the lymphatic drainage, then it would be expected that the reduced outflow of fluid would reduce the lymphatic reserve. If this were correct, then there would be an increased filtration rate and this would lead to an increase in the interstitial protein

concentration. However, it has been found that the interstitial protein concentration is inversely correlated with the increase in arm volume in BCRL.²⁸⁵ It has been suggested that the increase in interstitial pressure may cause a chronically raised afterload on smooth muscle function in the lymphatic walls. This would cause pump failure, as occurs in hypertensive cardiac failure.²⁸⁹

A sustained increase in venous pressure leads to an increase in capillary pressure and filtration rates. This translates into higher lymph flow, which alters drainage from the limb.¹⁴⁷ Bates *et al* found venous pressure was not elevated in patients with long-standing BCRL,²⁸⁵ but there is no evidence in the literature regarding venous pressures in patients prior to development of BCRL. If there is a constitutional problem in patients who develop BCRL then an elevated venous pressure may be contributing to development of this condition.

1.13.3 Lymphovenous communications

The lymphatic system terminates in the thoracic duct or lymphatic duct, and drains into the venous system. The rate of return of lymph to the blood via the lymphatic system can also be measured. An example is by measuring the accumulation of injected radiolabelled HIG in blood by taking serial blood samples from the antecubital vein of the contralateral arm. This would represent the rate at which the lymphatic system returns lymph to blood. The lymphatic clearance rates (measured as k_{depot}) have been shown to correlate with rates of accumulation in central blood.²⁹⁰

The existence of lymphovenous communications (LVCs) was first suggested by Threefoot in the 1960s and several animal and human studies have since shown the presence of LVCs, albeit in differing circumstances.^{58,291,292} Aboul-Enein *et al* investigated the presence of LVCs in breast cancer patients using direct intra-lymphatic infusion of labelled albumin followed by ipsilateral and contralateral blood sampling. In patients who did not develop BCRL, the radioisotope appeared in the blood stream earlier than in patients with BCRL or healthy controls. This suggested that in patients who did not develop BCRL, the presence of LVCs provided something akin to a protective effect.²⁹³ O'Mahony *et al* investigated the delivery of radio-labelled blood cells to lymphatic vessels using intradermal injection. Radiolabelled-erythrocytes were injected simultaneously into the hands of 4 normal subjects, intradermally on one side and subcutaneously on the other. Erythrocytes would only be able to access local blood through LVCs or needle trauma. Following intradermal injection, scintigraphy revealed abundant axillary activity, indicating erythrocyte transport up upper limb lymphatics. This was not observed following subcutaneous injection. When there was no evidence of cell-bound activity in ipsilateral blood, this indicated neither LVCs nor needle trauma. When similar studies were performed in four patients 3 months after axillary surgery, however, intradermally injected labelled erythrocytes were recovered bilaterally in central blood in one patient. Whether this confers any protection against BCRL is still not known. Of note, this patient was the only one in this group of patients who did not go on to develop BCRL, although this study was limited by a small number of participants.²⁹¹

1.14 Aim

This thesis aims to investigate and provide insight into the complex physiological processes that take place in women undergoing axillary lymph node surgery for breast cancer. Patients will be studied to see if the changes that take place are related to the development of BCRL. These studies will investigate if there are characteristics in some patients that may offer protection from BCRL. The main focus will be on whether patients have an increased susceptibility to BCRL, which manifests as a constitutional predisposition. There will be specific focus on the muscle lymph flow in the upper limb to see if there is any abnormality observed in those patients who subsequently develop BCRL. In addition to this, examining for the presence of lymphovenous communications in the upper limb will determine to what extent these exist in breast cancer patients and whether they confer a protective mechanism against BCRL. Finally, by studying the lower limb lymphatics in women who subsequently developed BCRL and comparing them with those who did not, it may be possible to assess if there is an underlying 'global' constitutional element that predisposes to the development of BCRL.

Hypotheses:

- I. Women who develop BCRL following breast cancer treatment have a higher lymph flow in the muscle compartment of both upper limbs prior to axillary lymph node surgery compared with women who do not develop BCRL.
- II. Lymphovenous communications are present in women who do not develop BCRL. These communications open as a rescue mechanism after axillary lymph node surgery and confer a level of protection against the development of BCRL

III. There is an inherent predisposition to lymphoedema, which manifests as a constitutional global lymphatic dysfunction in women who subsequently develop BCRL.

CHAPTER 2

2 General Methods

The studies presented in this thesis share a number of similar methodology that are described below. Study specific methods are detailed in the relevant individual chapters.

2.1 Overview of studies

Study 1 (Hypothesis I): *A prospective investigation of muscle lymph drainage in the upper limbs of breast cancer patients undergoing axillary lymph node dissection.* Lymphoscintigraphy was used to assess lymph flow and axillary nodal activity. Patients were studied pre- and post-operatively and followed up to see if they developed BCRL.

Study 2a (Hypothesis II): *A prospective investigation of the possible presence of lymphovenous communications in the upper limb of breast cancer patients undergoing axillary lymph node dissection.* Radiolabelled red blood cells were injected into the ipsilateral hand of patients and blood samples taken from both upper limbs to test for lymphovenous communications. Patients were studied pre- and post-operatively and followed up to see if they developed BCRL.

Study 2b (Hypothesis II): *An investigation of the possible presence of lymphovenous communications in the upper limb of breast cancer patients at least 3 years post-axillary lymph node surgery.* Radiolabelled red blood cells were injected

into the ipsilateral hand and blood samples taken from both upper limbs. Patients with and without BCRL were studied.

Study 3 (Hypothesis III): *An investigation of the lower limb lymphatic function in breast cancer patients with and without BCRL.* Lower limb lymphoscintigraphy was performed in breast cancer patients who had been treated with axillary lymph node dissection at least three years previously. Patients were divided into those who had developed BCRL and those who had not. Patients were assessed for the presence of a constitutional 'global' lymphatic dysfunction.

2.2 Ethics approval

Ethics approval was granted by the Outer North East London Research Ethics Committee (REC); reference number 09/H0701/112 (Study 1, 2a and 2b); reference number 11/LO/0892 (Study 3). All studies were approved by the Administration of Radioactive Substances Advisory Committee (ARSAC), reference number RPC 204/2035/25873. All study participants gave written informed consent.

2.3 Recruitment of patients

All study participants were women diagnosed with breast cancer. Studies 1 and 2a required prospective recruitment of such patients prior to any surgical treatment. These patients were screened from multi-disciplinary team (MDT) meetings at the Breast Unit at Guy's and St Thomas' NHS Foundation Trust (GSTT) and Brighton and Sussex University Hospitals NHS Trust (BSUH). For Studies 2b and 3, patients had to

be at least 3 years following initial breast cancer surgical treatment and these patients were screened from surgical and oncology follow-up clinics as well as from the lymphoedema clinics at GSTT and BSUH.

Potential participants were approached at outpatient clinics, by post or by telephone contact and then followed up. Patient information sheets were given to potential participants outlining the aims of the study and what was required. They were informed that the study could potentially be of no therapeutic benefit to them and would include exposure to radioactive material and blood sampling where necessary. All patients were given at least 24 hours to consider the study. Patients' General Practitioners were informed of their participation.

2.4 Power of studies and sample size

Study 1

The assessment of pre-operative muscle lymph drainage (k) has not previously been done, so there is nothing in the literature to guide a power calculation for this specific study. Based on previous work by Stanton *et al*²⁹⁴ looking at post-operative muscle k , it was thought that recruitment of 40 patients would be sufficient for this study.

Study 2a and 2b

These were observational studies and did not require a power calculation.

Study 3

In a recent quantitative lymphoscintigraphic study, the quantity of tracer accumulating in contralateral ilio-inguinal lymph nodes of patients with unilateral

lymphoedema and a normal contralateral limb was 70% (SD 38%) of the tracer accumulating in the nodes of patients with bilateral normal lymphoscintigraphy and no clinical evidence of lymphoedema in either limb.²⁹⁵ The SD in the latter group was 67% (mean = 100%). This is the closest scenario to the current proposal and if repeated would be significant, using an unpaired *t* test, at the 5% level for *n* = 30 (2 limbs analysed separately) in each group. No analogous data are available for *k*.

2.5 Clinical assessment and volume measurement of the upper limb

The ipsilateral upper limb was checked for the presence of oedema by carefully examining and comparing both upper limbs. The upper limbs were examined for decreased visibility of subcutaneous veins on the forearm and dorsum of the hand, smoothening or fullness of the medial elbow and distal upper limb contours, and increased skin and subcutis thickness by pinching the tissues between finger and thumb. Applying digital pressure for 60 seconds was the method used for testing for pitting oedema.

The Perometer 350S (Pero-system, Wuppertal, Germany) was used to measure total upper limb volume i.e. forearm and upper arm volume starting from the ulna styloid. The Perometer uses infrared light emitting diodes and corresponding sensors within a moveable square frame to measure upper limb size (Figure 3). The infrared transmitters are spaced 2.54mm apart and along two sides of the frame, and opposite, the photosensors are placed 1.27mm apart (Figure 4).



Figure 3 The Perometer (350S)

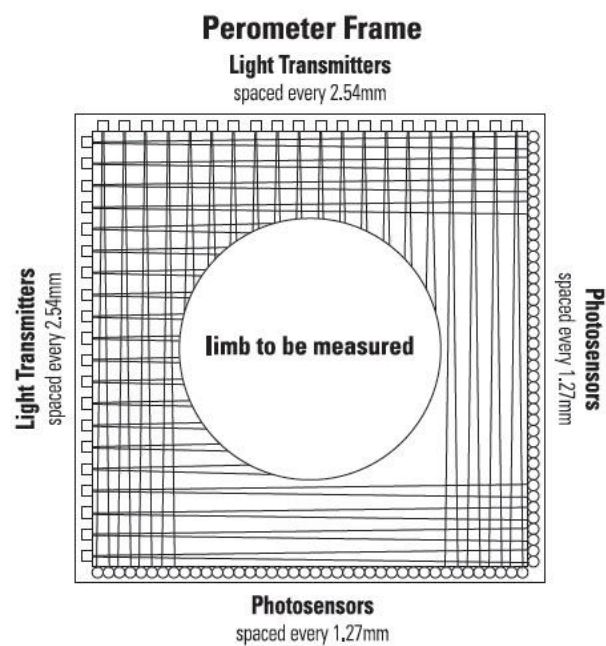


Figure 4 Diagrammatic cross-section of the Perometer measuring frame.

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The upper limb is placed inside the frame and the photosensors identify where the upper limb is obstructing the light beams. On moving the frame in the direction of

the arrow (Figure 3), pairs of diameters (in the vertical and horizontal plane) are measured every 5 mm and the derived volume is calculated by a computer. The shape (contour) of the upper limb is also recorded and displayed graphically (Figure 5); the circumference at any point and the volume between any desired limits can also be measured.^{161,226} The Perometer has been comprehensively evaluated and compared with other methods of limb measurement. It is a highly reproducible and convenient tool for the measurement of limb volume.²²⁸

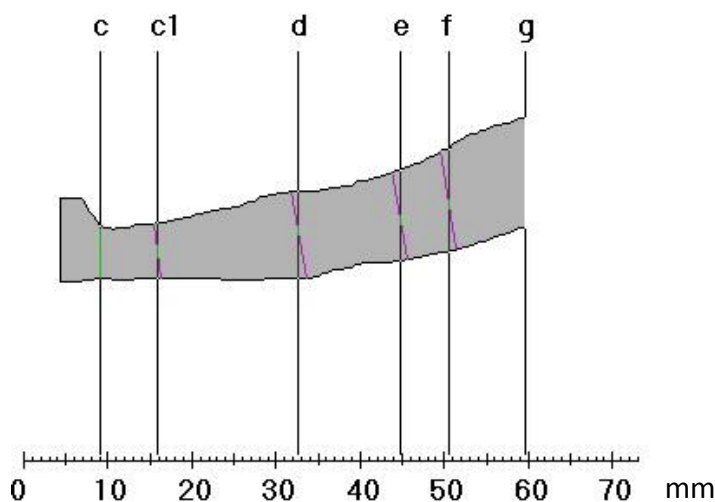


Figure 5 Two dimensional image of an upper limb (side-view) constructed by the computer.

2.6 Venous pressure measurement

A cannula (20G) or butterfly needle (20G) was inserted into a vein in the upper limb, usually in the antecubital fossa, flushed with sterile 0.9% saline and connected to a graduated manometer (central venous pressure set; Becton Dickinson, Oxford) (Figure 6). The manometer was positioned at the level of the manubriosternal angle (angle of Louis), approximately 5-6 cm above mid-right atrium level, and this level was adjacent to '0' on the scale by using the built-in rod and spirit level on the manometer.



Figure 6 Manometer system attached to a cannula in the antecubital vein

The sterile giving set and manometer tubing consisting of a 3-way tap and tubing were attached to the manometer. All lines were primed with saline. The manometer tubing was then attached to the patient's cannula. The manometer was opened to the patient and the height of the column of saline in the tubing fell and stabilised at a new lower level, which was recorded as the venous pressure (P_v) 5–6 cm above mid-right atrium level.

2.7 Lymphoscintigraphy

Lymphoscintigraphy, or isotope lymphography, is a well-established, safe and relatively non-invasive technique involving injection of a radiolabelled tracer in the distal aspect of a limb followed by subsequent imaging of the lymphatic vasculature with a gamma camera. This can provide information about the lymphatic anatomy

as well as lymphatic function. Clinical applications of lymphoscintigraphy are summarised in Table 15 ^{296,297}

General Application	Specifics
Diagnosis	Differentiation of lymphoedema from other causes of swelling
Assessment	Assessing the lymphatic pathway drainage
Identification	Identification of sentinel nodes in patients with cancer Identify degree and level of lymphatic dysfunction or delineate lymphatic malformations
Quantitation	Quantification of lymph flow

Table 15 Clinical applications of lymphoscintigraphy

Lymphoscintigraphy assesses three aspects of lymphatic function:

- The local rate of removal of interstitial macromolecules by small lymphatics over a few cm² of tissue
- Transport of label up the limb axis by larger lymphatics
- Transport through and retention by regional lymph nodes

Qualitative lymphoscintigraphy provides static images, which can show the gross anatomy of lymph vessels and lymph nodes, dilatation of lymphatic vessels, existence of collaterals and the presence of dermal backflow. There are alternative methods for assessing lymphatic function/structure under development, but lymphoscintigraphy is currently the method of choice. ²⁸⁶ Figure 7 shows lymphoscintigraphy images from a normal lower limb. There is symmetrical

accumulation in the ilio-inguinal lymph nodes, with no evidence of collaterals or dermal backflow.

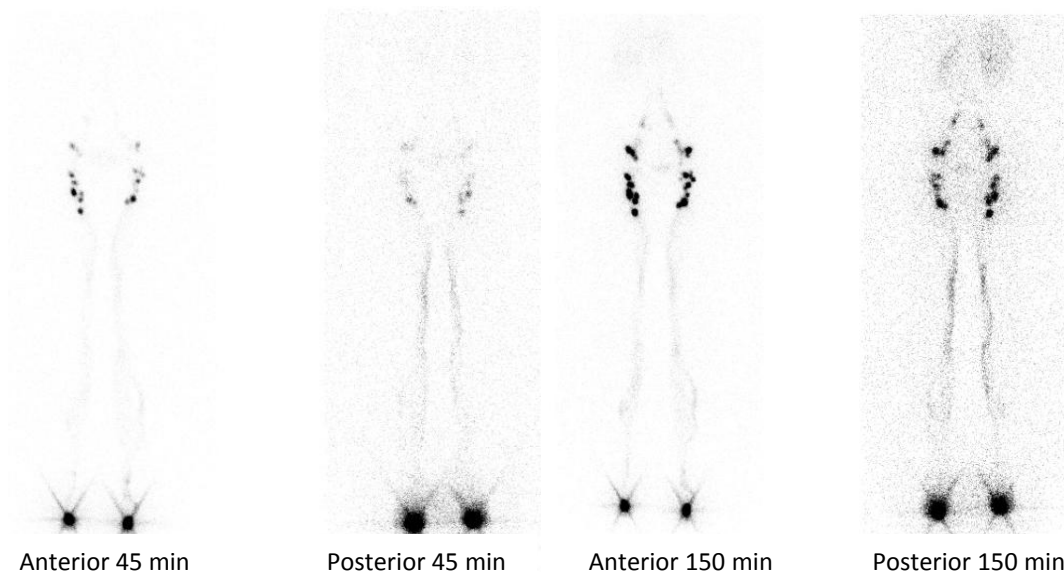


Figure 7 Normal lymphoscintigraphy of the lower limbs; anterior and posterior images at 45 and 150 min after injection.

2.8 Injection Site

Lymphoscintigraphy of the limbs involves distal injection of radiolabelled materials into the interstitial space. The choice of injection depth varies from intradermal (very rapid visualization of lymphatics), to subdermal and subcutaneous, where transit to the lymphatics is slower.²⁹⁷ A common injection site is the 2nd or 3rd webspace of the limb, although the dorsum of the hand or foot has also been used.²⁹⁷ Data suggest that the optimal route of injection may vary depending on the tracer used, with subcutaneous injection being optimal for colloidal agents.²⁹⁶ A study by O'Mahony *et al* (2004) concluded that intradermal (id) injection resulted in better image definition of lymph vessels immediately after injection when compared with subcutaneous (sc) injection, regardless of whether the

radiopharmaceutical was a colloid (^{99m}Tc -Nanocolloid) or macromolecule (^{99m}Tc -human IgG {HIG}).²⁹⁸ This finding was explained by the high concentration of lymphatic capillary plexuses in the dermis, which provides a high surface area for uptake of the agent and also the exertion of high interstitial pressure within the dermis, which increases the uptake of tracer by the lymphatics.²⁹⁸ Intradermal injection also delivers intact radiolabelled erythrocytes to lymphatic vessels and lymph nodes, which allows for the investigation of LVCs.²⁹⁹ In Study 2a and 2b, the ^{99m}Tc -erythrocytes were injected intradermally into the 2nd webspace of the hand of the ipsilateral side for lymphoscintigraphy images. The sample was prepared as outlined in Chapter 4 and Appendix 4.

Subfascial injection of radiotracers can be used for investigation of deep lymphatics.^{286,296} The injected radiolabelled tracers are removed by local clearance by the lymphatic capillary network, and with the use of the gamma camera, can be used to calculate lymph flow per unit tissue volume (k).²⁸⁶

2.9 Radiopharmaceuticals

There have been many radiopharmaceuticals evaluated for use in lymphoscintigraphy. They are generally classified as:

- Macromolecules
- Particulate structures

Examples of macromolecules include labelled dextrans, monoclonal antibodies and human serum albumin (HSA), whereas particulate structures include labelled

colloids or liposomes.³⁰⁰ It is thought that the injected agents travel through the lymphatic channels to the regional lymph node groups.³⁰⁰ They either enter the lymphatic capillaries passively, e.g. in the case of macromolecules, or through phagocytosis by macrophages and are transported within lymphatic channels, which occur with colloids.^{301,302}

Particles need to be of a certain size to be effective in lymphoscintigraphy – if particles are too small then they enter the blood stream and if they are too large then move slower through the interstitium. In animal studies, the optimal particle size has been estimated to be 5 nm for lymphatic drainage studies.³⁰² If smaller than this (0.05 nm – 5 nm) the particles usually leak into blood capillaries and therefore become unavailable to migrate through lymphatic channels.³⁰² Larger particles are prevented from entering the blood capillaries by a basement membrane and endothelial layer.^{300,302} Larger particles (> 100 nm) move very slowly from injection site to lymphatics leading to a lower accumulation in the lymph nodes, making them less suitable for lymphoscintigraphy.^{300,302} Large particles have been detected in venous blood immediately after subcutaneous injection, which is thought to be due to localised trauma from the injection site.^{296,301,302}

Optimal images of the lymphatic system would require the radiolabel to be taken up by lymphatics, retained with the nodes and not access blood vessels. The choice of radiolabel tracer (including its size and stability), the type and site of injection and the pharmacokinetics all influence the clinical information obtained. The

selection of the tracer depends on what information is required from the study.^{301,302} ^{198}Au -colloid was the first agent that was widely used, but as a significant amount of the dose remained at the injection site causing radiation burden, agents with more favourable characteristics were sought. ^{198}Au was replaced by the technetium-labelled tracers ($^{99\text{m}}\text{Tc}$).^{296,302} These have a short half-life of 6 hours and are ideal for imaging using the gamma camera.³⁰³ The main agents used are $^{99\text{m}}\text{Tc}$ -antimony sulphide colloid, $^{99\text{m}}\text{Tc}$ -sulfur colloid, $^{99\text{m}}\text{Tc}$ -albumin colloid, $^{99\text{m}}\text{Tc}$ -labelled HSA and $^{99\text{m}}\text{Tc}$ -Nanocolloid. The non-colloidal agents that have been used (labelled HSA, dextrans and human immunoglobulins) are soluble macromolecules, and have shown a more rapid uptake by lymphatics than colloids. However, as they are not particulate they are minimally retained by the lymph nodes and therefore less suitable for lymph node imaging.^{296,302}

In Europe, $^{99\text{m}}\text{Tc}$ -Nanocolloid (5-100 nm) is routinely used in the clinical setting.^{296,302} It is commonly available, has favourable properties, gives comparatively low radiation exposure and has an optimal gamma emission for imaging.³⁰² This therefore made $^{99\text{m}}\text{Tc}$ the radiopharmaceutical of choice for investigating the lymphatic system in these studies. Technetium (Tc) is a transition metal element and decays by emission of gamma radiation of 140 kilo electron-Volts (keV). Tc has a half-life of approximately 6 hours, allowing for sufficient time for imaging and does not result in an excessive radiation dose to the patient. It is also cheap and easy to produce, it can be easily bound to other molecules, it is non-toxic and associated with a low incidence of adverse reactions.³⁰⁴

2.9.1 Quality control performed by the Radiopharmacy Department

The stability of the bond between the radiolabel and the Nanocolloid is important because blood will clear any unbound or dissociating label at a rate that is two orders in magnitude faster than lymphatic clearance¹⁴⁷ which would cause k to be an overestimation of lymph flow. Radiochemical purity (RCP) is the radiochemical form that determines the bio-distribution of the radiopharmaceutical. Impurities will have different patterns of bio-distribution and these may obscure the diagnostic image obtained and alter the results of the investigation. A level of RCP accepted by the radiopharmacy department is 95%. At GSTT and BSUH the RCP of ^{99m}Tc was performed weekly.

2.9.2 Radiation risk and safety procedures

The dose of radiation was the minimum needed for each of the investigations. The Radiation Protection Adviser calculated the radiation dose in millisieverts (mSv) before submission to the REC. The average background radiation dose in the U.K. is 2.5mSv per annum. The studies involved a radiation dose of 0.1mSv per patient. The studies were done in accordance with the Ionising Radiation Medical Exposure Regulations (IRMER). At all times, transport, usage and storage regulations for ^{99m}Tc were adhered to.

2.9.3 Preparation of radiopharmaceutical

The radiopharmaceutical preparation for each study is explained below:

Study 1: ^{99m}Tc -Nanocoll was prepared by the addition of 1000 MBq sodium ^{99m}Tc -pertechnetate to a Nanocoll kit and diluting to 250 MBq/ml. A dose of 20

MBq of 0.2 ml ^{99m}Tc -Nanocoll was drawn into two 1 ml syringes with 25-gauge needles.

Study 2a and 2b: A blood sample was taken from the patient using a needle and heparinised syringe. The details of preparation of ^{99m}Tc -labelled red blood cells are described in Appendix 4. The ^{99m}Tc -labelled red blood cells were drawn into a 1 ml syringe using a 25-gauge needle.

Study 3: ^{99m}Tc -Nanocoll was prepared as described above. A dose of 20 MBq of 0.1 ml ^{99m}Tc -Nanocoll was drawn into two 1 ml syringes with 25-gauge needles.

2.10 Imaging with the gamma camera

The gamma camera is an imaging device for nuclear medicine and it consists of a large detector in front of which the patient is positioned.³⁰⁵ Gamma rays emitted from radiopharmaceuticals cause scintillation of the crystal within the gamma camera apparatus, which produces light that is detected by sensors and an image can be constructed based on this data (scintigraphy). A collimator modifies the gamma ray flux and is a lead plate consisting of an array of small holes. Only the gamma rays that travel along a hole axis will pass into the large NaI(Tl) scintillation crystal. Those gamma rays that approach at an oblique angle will hit the septa and be absorbed. Image resolution decreases with distance from the collimator, therefore resolution is better if the imaged organ is near the detector (Figure 8).³⁰⁶ Gamma cameras have separate collimators for the different energy of radionuclides used and the type of investigation to be performed. For these studies, a low energy, high-resolution collimator is ideal which detects gamma photon energy of <140 keV.

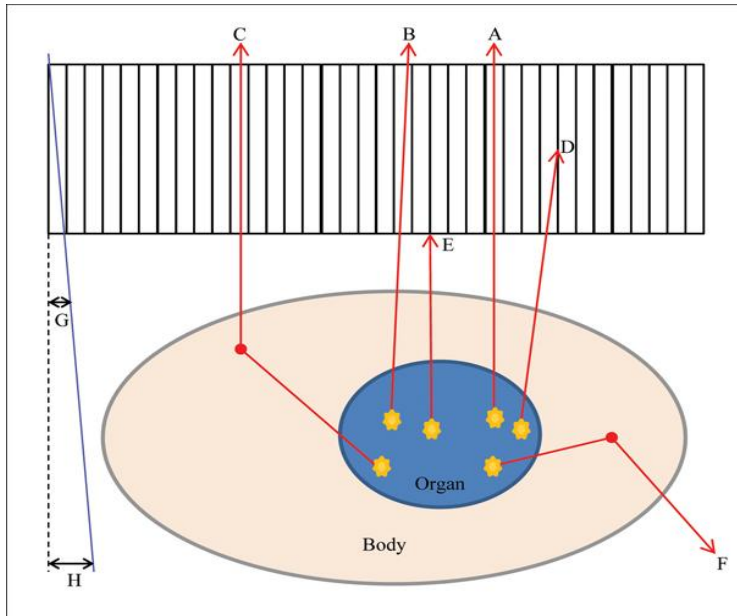


Figure 8 The collimator

Photons perpendicular to the plane (A) pass through the collimator to interact with the detector. B would also be registered. C-F events would not be included in the final image. G-H shows how increasing the distance would increase error.³⁰⁶

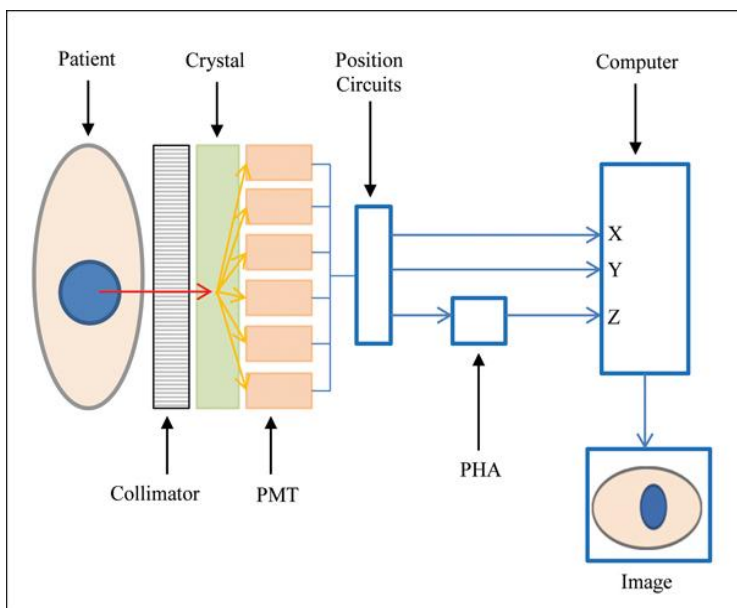


Figure 9 Schematic diagram of the gamma camera.³⁰⁶

PMT, photomultiplier tubes; PHA, pulse height analyser

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The detector assembly is responsible for converting the gamma rays from the patient into a form, which allows visible images to be produced. The first stage is when the gamma radiation from the patient is absorbed by the scintillation crystal and converted into ultraviolet light. The second stage involves transformation of these light signals into electronic signals by an array of photomultiplier tubes (PMT). Position circuitry provides x and y coordinates for the incident and this coordinate is registered if a z signal is received. The z signal represents the energy deposited in the crystal by the gamma ray.³⁰⁷ The pulse height analyser (PHA) tests whether the energy of the gamma ray is within the range expected for the specific radionuclide being imaged (Figure 9).

A gamma camera can provide the following measures:

- The rate of disappearance of radioactivity, which can be quantified from a region of interest (ROI) that encompasses the entire depot and a surrounding zone of unlabelled tissue.
- The increase in radioactivity over proximal limb segments assesses the transport along large contractile vessels. Lymphoedema characteristically shows 'dermal backflow' which is re-routing of the tracer from the main trunks into collateral lymphatics of the proximal skin
- Lymph node activity by assessing the arrival and retention of the tracer at the regional lymph nodes.²⁸⁶

Imaging was performed with a single-headed camera (Symbia Gamma Camera, Germany or Sopha Medical Camera, France) with a low-energy high resolution collimator, which have been used for similar studies of this nature.³⁰⁸ (Table 16) A 256 x 256 matrix was used for all images with one pixel representing 0.238 x 0.238 cm. Images were analysed using the HERMES imaging software system.

Manufacturer and model	Detector	Field of view	Collimator
Siemens Symbia	15mm NaI	53 x 39 cm	Low energy, high resolution
Sopha Medical DSX	13mm NaI	45 x 40 cm	Low energy, high resolution

Table 16 Gamma camera details

2.11 Measurement of lymph drainage constant k

Measurement of the rate of clearance of the radiolabelled Nanocolloid was the method used for measuring lymph flow quantitatively. An explanation of the theory relating k to lymph flow is given in Appendix 3. Investigation of factors known to influence lymph flow and capillary filtration rate, e.g. exercise, adrenaline and inflammation have produced expected changes in k .^{286,288,309-311} k is therefore the best method currently used for quantitative assessment of lymphatic clearance.

k was measured by calculating the rate of disappearance of radioactivity from the depot site and this indicated the rate of removal of the protein by lymphatic drainage. Local clearance rates are calculated using the region of interest (ROI).

The counts in the ROI begin to fall as the depot is cleared by the lymphatics, as well as secondary to decay of the tracer. A plot of \log_e of residual counts (corrected for radioactive decay and background activity) against time should be a negative mono-exponential slope. The slope of the plot is the lymphatic rate constant (k), which provides an estimate of the local lymph flow per unit volume of distribution of radiotracer in the interstitial fluid. The rate constant (min^{-1}) has the units of fraction of tissue volume cleared of solute per unit time.²⁸⁶

CHAPTER 3

3 Study 1: An investigation into the muscle lymph drainage of the upper limb

3.1 Introduction

In breast cancer patients, with and without BCRL, k has been previously measured in the forearm epifascial compartment (subcutis or skin), forearm subfascial compartment (skeletal muscle) and hand (subcutis).^{288,294,308,310} Muscle lymph drainage has been shown to correlate with degree of limb swelling in BCRL, unlike subcutis drainage, which has no correlation, thereby making this a more accurate measurement tool.^{308,310}

In a previous study, k for ^{99m}Tc -human polyclonal IgG (^{99m}Tc -HIG) was measured bilaterally in forearm muscle in 43 women at 7 and 30 months after surgery for breast cancer.²⁹⁴ At 7 months after surgery none of the patients had BCRL. By 30 months 19% of patients ($n = 7$) had developed mild BCRL, the ipsilateral upper limb being $5.8 \pm 2.0\%$ larger than the contralateral upper limb. When the BCRL-destined (pre-BCRL) and non-BCRL groups were compared at 7 months, k was found to be significantly higher in the pre-BCRL group. Muscle k was found to be 22% higher in the muscle of the ipsilateral upper limb of the pre-BCRL group than in the non-BCRL group. Moreover there was a similar, significant difference in the contralateral upper limb k values, and also in subcutis k values on both sides. This led to the 'high

filterers' hypothesis, namely that some women have a constitutively high rate of capillary fluid filtration and hence high fluid loading of the lymphatic system, which predisposes them to secondary lymphoedema, independent of the number of axillary nodes removed.²⁹⁴

The above study did not address the question of whether the high fluid loading was innate, i.e. whether it existed prior to surgery, or whether it was the response of a subset of patients (those who subsequently developed BCRL), to axillary lymph node surgery. Therefore, the purpose of this prospective study was to address this distinction, with the aim of studying patients due to undergo axillary lymph node surgery prior to any surgical intervention and to determine if there was any difference in patients who developed BCRL compared with those who did not. Local lymph flow (k) was measured in the upper limbs of newly diagnosed breast cancer patients.

3.2 Study Aim

The aim of this study is to test the hypothesis that women who develop BCRL subsequent to breast cancer treatment have higher lymph flow in the muscle compartment of both upper limbs prior to axillary lymph node surgery compared with women who do not develop BCRL. In addition, a further aim of this study is to determine whether axillary lymph node surgery substantially impairs lymph drainage from the forearm muscle compartment in the short term.

3.3 Study Design

This was a prospective observational study using quantitative lymphoscintigraphy (QL) to investigate the local lymph flow (k) in the subfascial compartment of the forearms of newly diagnosed breast cancer patients. In all, 38 patients had a diagnosis of unilateral breast cancer and had not previously had any axillary lymph node surgery. Both upper limbs were studied pre-operatively and post-operatively. The study of both the ipsilateral and contralateral side provided a control value for k in each patient. The technique has previously been validated by the significant correlation ($r = 0.51$, $p < 0.01$) between measurements in the two upper limbs of the same patient, i.e. side-to-side comparison; repeated methodological measurements on the same limb would be unethical.²⁹⁰ Patients were studied both prior to surgery and approximately 4-6 weeks after axillary lymph node surgery. The post-operative study timing was kept flexible within this time to allow patients time to recover from their surgery. Patients were then followed up to see which patients subsequently developed BCRL and assessed for any correlation between lymph flow and the onset of BCRL. This enabled establishment of whether the lymph drainage rate was higher before the axillary lymph node surgical intervention in the group that subsequently developed BCRL. Lymph drainage was measured in forearm skeletal muscle rather than subcutis because muscle capillary filtration rate, and hence lymph flow, is higher than in the subcutis, and thus contributes the majority of total upper limb lymph flow. ^{99m}Tc-HIG is no longer available so the radioisotope used was ^{99m}Tc-Nanocoll. This has a particle size of approximately 80 nm, which is too large a particle to be cleared into the blood, but small enough to

be cleared by the lymphatic system, as confirmed by its appearance in upper limb lymphatics and axillary lymph nodes.^{286,298}

3.4 Methods

3.4.1 Recruitment of patients

Patients recently diagnosed with invasive breast cancer and due to undergo 4NAS at Brighton and Sussex University Hospitals NHS Trust (BSUH) or level II/III ALND at BSUH or Guy's and St Thomas' NHS Foundation Trust (GSTT) were recruited from the Breast Clinic. As per the power calculation (section 2.4), pre-operative muscle lymph drainage (k) has not previously been studied, so there was nothing in the literature to guide the power calculation for this specific study. Based on previous work by Stanton *et al*²⁹⁴ examining post-operative muscle k , it was thought that recruitment of 40 patients undergoing axillary lymph node dissection (ALND) would be sufficient for this study. With an incidence of BCRL of approximately 20-25% in patients undergoing ALND, this number should have given sufficient power to the study. However, there were problems with recruitment as the patients were concerned with regard to the risk of BCRL development if they were to have injections into the ipsilateral upper limb after axillary surgery. As a consequence of this, it was decided that it would be appropriate to include patients due to undergo 4NAS, accepting that patients undergoing this procedure have a lower risk of developing BCRL (approximately 5%³¹²). In all, 210 patients at BSUH were screened, of whom 23 gave consent to take part in the study (20 due to undergo 4NAS and 3 ALND); 115 patients due to undergo ALND at GSTT were screened, of whom 15 gave

consent to take part in this study. Following written informed consent, patients were studied in the Nuclear Medicine departments at either BSUH or GSTT. Patients attended on three occasions: pre-operatively, two to six weeks post-operatively and 1 year post-operatively (follow-up visit). On the pre-operative and post-operative visits, the following procedures were performed:

- (i) Clinical assessment for the presence of lymphoedema;
- (ii) Upper limb volume measurement;
- (iii) Muscle lymph drainage estimation by quantitative lymphoscintigraphy;
- (iv) Axillary lymph node gamma camera imaging.

Venous pressure was only measured pre-operatively and only clinical and upper limb volume assessments were performed at the follow-up visit.

3.4.2 Injection site and patient positioning

The injection site was the thickest and fleshiest part of the proximal forearm. This site was identified on the ipsilateral upper limb and two short lines were drawn transversely on the forearm on either side of the selected site, and two short lines longitudinally in the form of 'cross-hairs' (Figure 10).

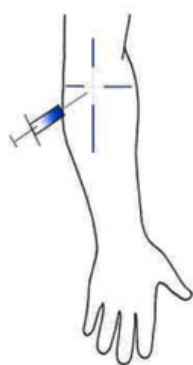


Figure 10 Diagram showing the position of the injection site

The position was calculated by measuring the distance from this site to the end of the fully extended middle finger and also measuring its distance from the mid-line of the forearm (in mm). These measurements were used to calculate the injection site on the contralateral upper limb and for injections on subsequent visits. Once the injection site was identified, the patient acclimatised for at least 20 minutes and was then seated with both upper limbs resting on a table. The palms were placed together with fingers and thenar eminences touching, i.e. the forearms were semi-pronated. A single-headed camera (Symbia Gamma Camera, Siemens, Germany or Sopha Medical Camera, France) with a low-energy high-resolution collimator was positioned ~ 20 cm above the upper limbs. The ipsilateral upper limb was injected first. The skin, subcutis and muscle of the forearm were pinched up between thumb and forefinger and the 25G needle was inserted perpendicularly to its full length (25mm, or 1 inch). The grip was relaxed and the ^{99m}Tc -Nanocoll (~20 MBq) in 0.2ml (G.E. Ltd., Amersham, Bucks, UK) was injected intramuscularly in each

forearm at the selected site over 2-3 seconds and the needle withdrawn. The opposite upper limb was injected similarly.

3.4.3 Image acquisition

The scans were conducted in a temperature-controlled room. Skin temperature (T_{sk}) was recorded with a thermometer using thermistor probes (4600 Precision Thermometer, Measurement Specialties Inc., USA). The camera height was kept the same for each acquisition and the upper limbs were repositioned as closely as possible. An outline of the patient's upper limbs was drawn to on the Incopad to facilitate placement of the upper limbs (Figure 11A). The first forearm acquisition was obtained approximately 2 minutes post-injection. Each acquisition took 60 seconds. Subsequent acquisitions were performed at 15, 30, 45, 60, 90, 120, 150 and 180 minutes post-injection.



Figure 11 The Siemens Symbia gamma camera

(A) Forearm imaging position (B) Upper arm and axilla imaging position.

The upper arms and axillae were imaged less frequently than the forearms. For this, the table was removed and the patient was seated in front of vertically oriented camera head with shoulders as close as possible to the camera for ventral viewing (Figure 11B). It was ensured that both entire upper arms and axillae were in the field of view. A 180 sec acquisition of images was performed at 50, 125 and 185 minutes post-injection. An outline acquisition using the cobalt-57 pen marker was performed at 130 minutes post-injection, which involved drawing around the shoulder starting at the acromion process continuing downwards to the lower limit of the camera field of view, and then up again on the inside of the upper limb. All images were marked in the top right-hand corner with the cobalt-57 pen marker. The patient was allowed to sit in the waiting room in between acquisitions.

3.5 Image analysis

3.5.1 Calculation of the lymphatic removal rate constant (k)

The clearance of radiotracer from the interstitial depot was quantified in a circular region of interest (ROI) of area 37.5cm^2 , which encompassed the entire forearm depot. The fraction of the counts remaining in the depot (corrected for radionuclide physical decay) was plotted semi-logarithmically. The slope of the linear plot of \log_e fraction *versus* time gave the fraction of tracer removed per minute. Multiplying by 100 gave k in units of % tracer removed per min. This equals the local lymph flow (ml/min) per 100ml of interstitial fluid in which the tracer was distributed. The theory relating k to lymph flow has been explained in section 1.13.1 and discussed extensively in the literature.^{286,288,309-311}

3.5.2 Axillary lymph node activity

The arrival and retention of the radioactive tracer in the regional lymph nodes was quantified by drawing a ROI over the axillae. After correcting for decay and background activity, the activity in the axillary and supraclavicular nodes was expressed as a percentage of the counts from the first acquisition of the depot at each time-point. Data were acquired for 35 patients pre-operatively and 27 patients post-operatively.

3.6 Statistical analysis

Results are shown as the mean \pm standard deviation (SD). Group comparisons were made using paired and unpaired *t* tests and 2-way ANOVA. The normal distribution of data was first ascertained by the Kolmogorov-Smirnov test. Linear regression analysis was used to analyse the mono-exponential slope of the depot clearance plot. Fisher's exact test was used for categorical data due to the small sample sizes. Pearson's *r* test was used for correlation. Analysis was performed using GraphPad Prism (version 6; San Diego, CA, USA). A *p* value of ≤ 0.05 was regarded as statistically significant.

3.7 Results

Patients who subsequently developed BCRL are referred to as 'pre-BCRL' patients in the pre-operative and post-operative visits described below.

3.7.1 Patient data

Pre-operative measurements were performed in 38 women, of whom 33 attended the post-operative study and 31 patients attended the follow-up visit. One patient

died prior to her follow-up visit. The mean age of patients at the time of surgery was 57 ± 9 years (range: 32-75 years) and the body mass index (BMI) was 29.0 ± 6.7 kg/m². Clinical, surgical and pathological details are summarised in Table 17. The intervals between surgery and subsequent assessments were 8 ± 6 weeks (post-operative visit) and 58 ± 9 weeks (follow-up visit). Seven patients developed BCRL using the clinical criteria for diagnosis at 6 ± 6 months after surgery, giving an overall incidence of 18%, based on clinical examination. Three patients developed BCRL in their dominant arm and four in their non-dominant arm. In all, 35/38 patients (92%) were right hand-dominant and 3/38 (8%) left hand-dominant. A total of 16/38 (42%) of patients had surgery to their dominant side.

The age of the BCRL patients and the non-BCRL patients did not differ significantly ($p = 0.46$, unpaired t test); see Table 18. The pre-operative body mass index (BMI) was not significantly different between the pre-BCRL and non-BCRL groups (28.2 ± 6.5 kg/m² vs. 32.4 ± 7.0 kg/m², $p = 0.18$). There was correlation between BMI and ipsilateral arm volume ($r = 0.73$, $p < 0.0001$, **Figure 12**). The mean did not change significantly over the course of three visits ($p = 0.08$, repeated measures one-way ANOVA). There was, however, significant correlation between the increase/decrease in individual BMI from pre-operative to post-operative visits and the increase/decrease in ipsilateral upper limb volume ($r = 0.36$, $p = 0.04$, Pearson's r test, Figure 13). This correlation had ceased by the follow-up visit ($r = 0.18$, $p = 0.33$).

Patient ID	Age (yrs)	Breast surgery	Axillary surgery	Number of lymph nodes removed (no. positive nodes)		Histology			
						Grade	Type	Size (mm)	ER status
001B	56	WLE	ANS	4	(0)	3	IDC	40	+
002B	67	WLE	ANC	17	(13)	2	IDC	18	+
003B*	75	WLE	ANS	8	(0)	2	IDC	18	+
004B	66	WLE	ANC	11	(3)	2	IDC	54	+
005B	61	WLE	ANS	7	(0)	2	IDC	21	+
006B	59	WLE	ANS	5	(0)	2	IDC	12	+
007B	76	WLE	ANS	4	(0)	1	IDC	8	+
008B	55	Mx	ANS	5	(2)	2	IDC	15,18	+
009B	51	WLE	ANS	7	(0)	1	IDC	15	+
010B	47	Mx	ANC	9	(9)	2	ILC	50	+
011B	51	WLE	ANS	9	(2)	2	IDC	6	+
012B	52	WLE	ANS	7	(0)	2	ILC	95	+
013B	69	WLE	ANS	6	(0)	2	IDC	29	+
014B	51	WLE	ANS	6	(0)	2	IDC& ILC	20,6	+
015B	49	WLE	ANS	2	(0)	3	IDC	11	+
016B	65	WLE	ANS	5	(0)	2	IDC	22	+
017B	66	WLE	ANS	4	(0)	2	IDC	20	+
018B	50	WLE	ANS	10	(0)	1	IDC	12,6,2	+
019B	60	WLE	ANS	8	(0)	2	ILC	20	+
020B	56	WLE	ANS	5	(0)	2	IDC	11	+
021B	45	WLE	ANS	4	(0)	1	IDC	15	+
022B	57	WLE	ANS	6	(0)	2	IDC	15	+
023B	64	WLE	ANS	4	(0)	3	IDC	16	-
007G	46	Mx	ANC	13	(1)	3	IDC	30	+
008G*	56	Mx	ANC	5	(0)	2	ILC	17,11	+
009G	51	Mx	ANC	8	(1)	2	IDC	28	+
010G	71	Mx	ANC	13	(2)	2	IDC	44	-
011G	66	Mx	ANC	20	(11)	3	IDC	30	-
012G*	49	WLE	ANC	9	(1)	3	IDC	10	+
013G*	67	WLE	ANC	5	(0)	3	IDC	0	-
014G	52	WLE	ANC	7	(1)	2	IDC	12	+
015G*	62	WLE	ANC	8	(3)	2	ILC	31	+
016G	44	Mx	ANC	29	(28)	2	ILC	28	+
017G*	52	Mx	ANC	4	(3)	2	IDC	120	+
018G*	53	WLE	ANC	15	(2)	2	IDC	29	+
019G	57	WLE	ANC	14	(1)	2	IDC & ILC	17	+
020G	33	WLE	ANC	19	(1)	2	IDC	14	+
024G	49	Mx	ANC	15	(7)	3	IDC	17	-

Table 17 Clinical, surgical and histopathological details of patients

*patients who developed BCRL; ANS, axillary node sampling; ANC, axillary clearance surgery; ER, oestrogen receptor; WLE, wide local excision; Mx, mastectomy; IDC, invasive ductal carcinoma no special type; ILC, invasive lobular carcinoma.

	BCRL (<i>n</i> = 7)	Non-BCRL (<i>n</i> = 31)	<i>p</i>
Age (years)	59 ± 9	56 ± 9	0.46
BMI (kg/m ²)	32.4 ± 7.0	28.2 ± 6.5	0.18
Nodes removed	7.7 ± 3.7	9.1 ± 6.0	0.44
Positive nodes	1.3 ± 1.4	2.7 ± 5.8	0.25
ANC surgery	6 (86%)	12 (39%)	0.038
ANS surgery	1 (14%)	19 (61%)	0.038
Wide local excision	5 (71%)	23 (74%)	1.0
Mastectomy	2 (29%)	8 (26%)	1.0
Chemotherapy	3 (43%)	9 (29%)	0.66
Neo-adjuvant chemotherapy	4 (57%)	10 (32%)	0.39
SCF radiotherapy	4 (57%)	8 (26%)	0.18

Table 18 Comparison between pre-BCRL and non-BCRL groups

Mean ± SD. BMI, body mass index; ANC, axillary clearance surgery; ANS, axillary node sample; SCF; supraclavicular fossa

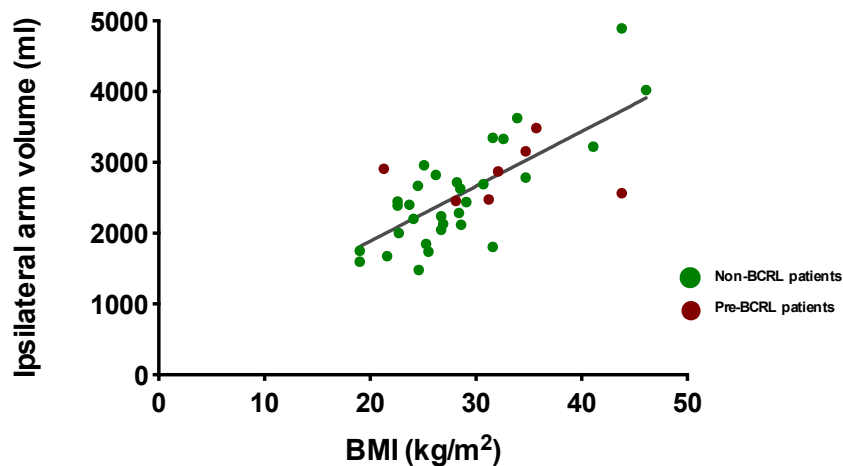


Figure 12 Correlation of BMI (kg/m²) and ipsilateral arm volume (ml) of patients at the pre-operative visit.

BMI, body mass index; $r = 0.73$, $p < 0.0001$.

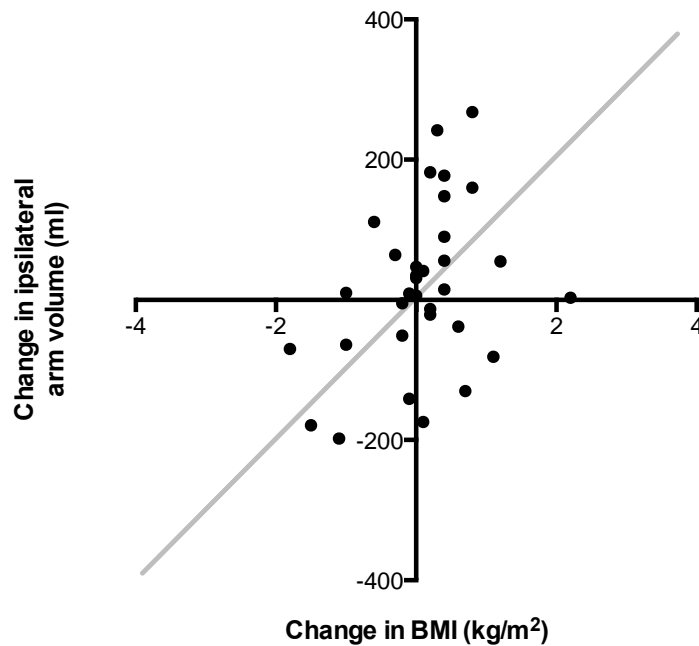


Figure 13 Change in BMI (kg/m²) plotted against change in ipsilateral upper limb volume (ml) Each point corresponds to a patient. There was significant correlation between the change in BMI from pre-operative to post-operative visits and the change in ipsilateral upper limb volume ($r = 0.36$, $p = 0.04$, Pearson's r test).

There were no differences between groups in the number of nodes (and the number of positive nodes) removed or the proportion of patients undergoing systemic therapy. Axillary lymph node clearance surgery rather than axillary node sampling appeared to be a significant risk factor for BCRL ($p = 0.038$, unpaired t test). The incidence of BCRL was 33% (6/18) in patients undergoing ANC compared with 5% (1/20) in those undergoing ANS. The average number of nodes removed in patients undergoing ANC was 12.1 ± 6.4 (median 11, range 4 -29) compared with 6.0 ± 2.3 in patients undergoing ANS (median 5.5 range 2 – 11). The extent of the breast surgery and whether or not radiotherapy was administered to the

supraclavicular fossa did not differ between the BCRL and non-BCRL groups. (Table 18)

3.7.2 Upper limb volume changes

Upper limb volume changes are summarised in Table 19. Although pre-BCRL patients tended to have larger upper limb volumes bilaterally than non-BCRL patients, the differences were not statistically significant (ipsilateral $p = 0.29$; contralateral $p = 0.37$, $p = 0.16$, unpaired t test). On pooling pre-operative ipsilateral and contralateral upper limb volume data for the pre-BCRL group ($n = 14$) and comparing with the non-BCRL group ($n = 62$), there was also no significant difference ($p = 0.16$, unpaired t test). There was no significant difference in BMI between the two groups.

At the post-operative visit, the ipsilateral upper limb of the pre-BCRL patients was $5.6 \pm 2.7\%$ (140 ± 95 ml) larger than the contralateral upper limb ($n = 6$, $p = 0.004$, paired t test). Three of these patients demonstrated clinical signs of BCRL in the ipsilateral upper limb, which would demonstrate an initial swelling of the upper limb, with a mean 6.7% excess volume. The excess volume was 5.1% in those with no clinical swelling.

At the follow-up visit, 7 patients were diagnosed with BCRL, based on the previously defined clinical criteria. The Perometer showed an increase in BCRL ipsilateral upper limb volume, relative to the contralateral upper limb, of only 5% (135 ± 275 ml), a difference that was not statistically significant ($p = 0.24$, paired t test). In marked contrast to the BCRL group, both upper limb volumes in non-BCRL patients were

remarkably constant at all time points, including the post-operative period (Table 19).

3.7.3 Pre-operative muscle lymph drainage rates in pre-BCRL patients compared with non-BCRL patients (Table 20)

^{99m}Tc -Nanocoll clearance from the forearm injection depot was evident from the earliest time points, without the initial plateau previously reported for ^{99m}Tc -HIG clearance.²⁸⁶ The median (interquartile) correlation coefficient of the exponential fit to the data points ($n = 142$) (both sides and both visits pooled) was 0.95 (0.93-0.96).

In the pre-operative patients there was a significant correlation between ipsilateral k ($k_{\text{ipsilateral}}$) and contralateral k ($k_{\text{contralateral}}$), as expected for a valid measure of fluid drainage ($r = 0.52$, $p = 0.001$, Pearson's r test). Likewise, in the subgroup of 7 patients who later developed BCRL the pre-operative $k_{\text{ipsilateral}}$ (0.0906 ± 0.0207 %/min) did not differ significantly from $k_{\text{contralateral}}$ (0.1017 ± 0.0442 %/min, $p = 0.38$, paired t test). To test the hypothesis of constitutively high fluid turnover rates in pre-BCRL patients, their pooled k values ($n = 14$) were compared with those of non-BCRL patients ($n = 62$) (Figure 14). On average the pre-BCRL patients had a 16.5% higher k (i.e. muscle lymph drainage rate) than the non-BCRL patients (0.0962 ± 0.0337 %/min vs. 0.0826 ± 0.0188 %/min, respectively). The higher k of the pre-BCRL group was statistically significant ($p = 0.042$, unpaired t test). This finding was in line with the 'high filterers' hypothesis. However, due to the small numbers of patients in the pre-BCRL group ($n = 7$), there is the possibility of a type 1 error. This would make the difference between the pre-BCRL and non-BCRL group less significant.

In the post-operative group the BCRL patients showed no significant difference between $k_{\text{ipsilateral}}$ (0.0772 ± 0.0231 %/min) and $k_{\text{contralateral}}$ (0.0828 ± 0.0033 %/min, $p = 0.59$). The non-BCRL patients similarly showed no significant differences between upper limbs. $k_{\text{ipsilateral}}$ for the BCRL group (0.0772 ± 0.0231 %/min) was not significantly lower than that for the non-BCRL group (0.0849 ± 0.0178 %/min, $p = 0.47$, unpaired t test). Two-way ANOVA was performed to test whether k was affected significantly by surgery (pre- vs. post-operative k values) or by side (ipsilateral vs. contralateral k values). The analysis indicated that k was not significantly affected by the surgery (pre- vs. post-operative $p = 0.31$) or side (ipsilateral vs. contralateral $p = 0.43$). The most marked difference, a fall in mean $k_{\text{ipsilateral}}$ from 0.0906 ± 0.021 %/min pre-operatively to 0.0772 ± 0.023 %/min post-operatively, was not significant ($p = 0.38$, paired t test). The change in $k_{\text{ipsilateral}}$ was not dependent on whether patients had ANS or ANC ($p = 0.90$ and $p = 0.49$ respectively, paired t test). The results thus indicated that axillary lymph node surgery did not alter lymphatic drainage from forearm muscle (**Figure 15**).

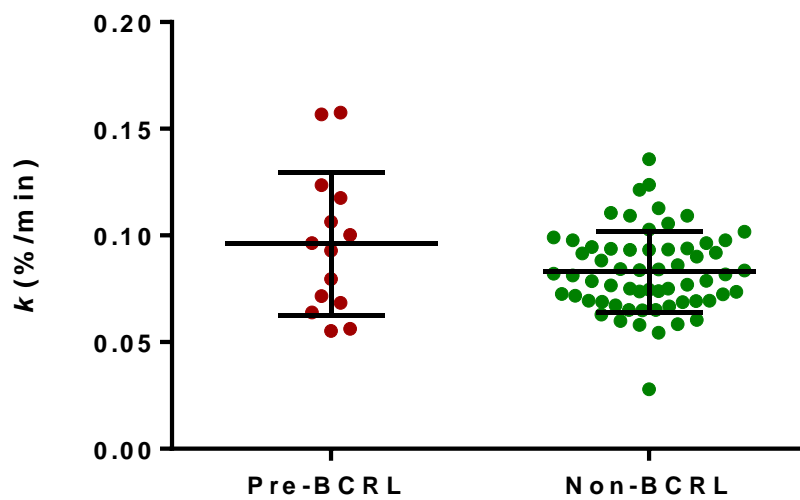


Figure 14 Muscle lymph drainage rates (k) at the pre-operative stage in patients who later developed BCRL (0.0962 ± 0.034 %/min) compared with those who did not develop BCRL (0.0830 ± 0.019 %/min). Pooled ipsilateral and contralateral k values ($p = 0.042$, $n = 14, 62$; unpaired t test).

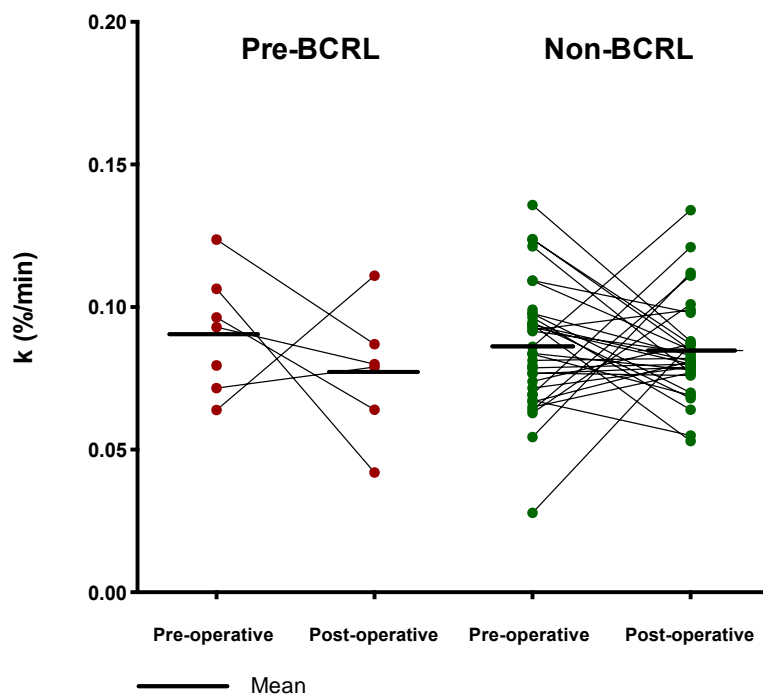


Figure 15 Joined plots for pre- and post-operative k measured in the subfascial compartment of the ipsilateral upper limb

3.7.4 Axillary activity

The axillary activity as a percentage of the injected activity increased over time in nearly all patients (Figure 16). In the non-BCRL patients there was a smooth, curvilinear increase over 185 min pre-operatively ($n = 28$), and the marginally reduced accumulation post-operatively was not statistically significant in either upper limb ($n = 23$) (ipsilateral upper limb $p = 0.19$, contralateral upper limb $p = 0.15$; t test). In the BCRL patients the ipsilateral pre-operative accumulation pattern was more variable ($n = 4$) and the contralateral axilla showed a more pronounced post-operative reduction in accumulation rate. However, these patient numbers are small so the significance of the observation is limited (**Figure 17**).

The axillary activity was not significantly different pre- and post-surgery in patients undergoing ANS or ANC ($p = 0.10$ and $p = 0.11$ respectively, paired t test). The axillary accumulation data indicated that surgery did not cause a major change in axillary activity, despite patients undergoing surgical excision of axillary lymph nodes.

3.7.5 Relationship between k and other variables

There were no significant correlations between $k_{\text{ipsilateral}}$ and upper limb volume in either the BCRL or non-BCRL group ($r \leq 0.1$; $p > 0.1$). Further analysis tested the relationships between change in $k_{\text{ipsilateral}}$ (post-operative vs. pre-operative) and various potential risk factors namely age, BMI and size of tumour. The average number of nodes removed was different between the groups; ANC 12.3 ± 6.4 ($n = 18$) and ANS was 5.8 ± 2.1 ($n = 20$). However, when comparing k between the

patients undergoing ANS or ANC or correlating k with the number of lymph nodes removed, k was not significantly different when comparing either the type of surgery performed or the number of nodes removed, indicating that the extent of surgery was not a confounding variable in this study. In the BCRL group there was no association between the change in $k_{\text{ipsilateral}}$ and age ($r = -0.12$; $p > 0.1$), BMI ($r = -0.71$; $p > 0.1$), the number of lymph nodes resected ($r = -0.03$; $p > 0.1$), type of surgery ($r = -0.07$; $p > 0.1$) or size of tumour ($r = 0.14$; $p > 0.1$). Similarly, there was no association between change in $k_{\text{ipsilateral}}$ and these factors in the non-BCRL group, with $p > 0.1$ for all categories. The time between surgery and post-operative visits was variable depending on when was convenient for the patient to attend. Although this ranged from 2 to 19 weeks, the timing of the postoperative visit was not related to change in $k_{\text{ipsilateral}}$. Venous pressure was measured in 16 patients (11.9 ± 2.5 cm H₂O) and there was no correlation between k and venous pressure ($r = 0.4$; $p > 0.1$). Upper limb dominance was not found to be a significant factor in the rate of muscle lymph drainage. No difference was found between the dominant and non-dominant upper limb k values either pre-operatively or post-operatively.

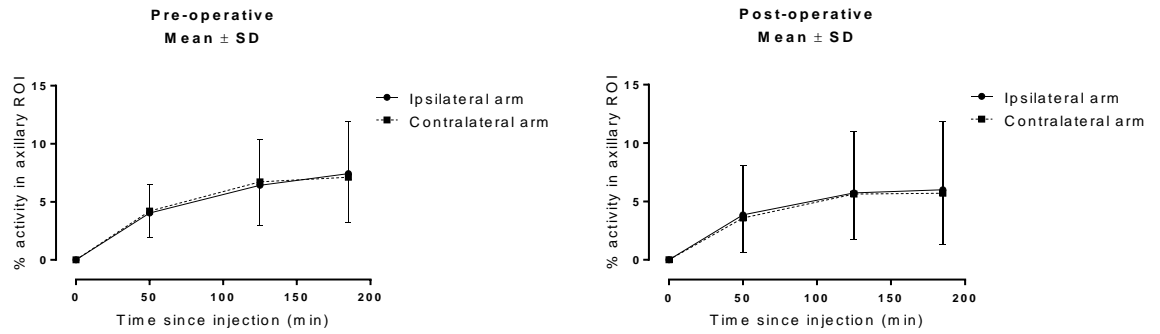


Figure 16 Mean axillary activity as percentage of depot injection in all patients (both non-BCRL and pre-BCRL), pre-operatively ($n = 35$) and post-operatively ($n = 27$).

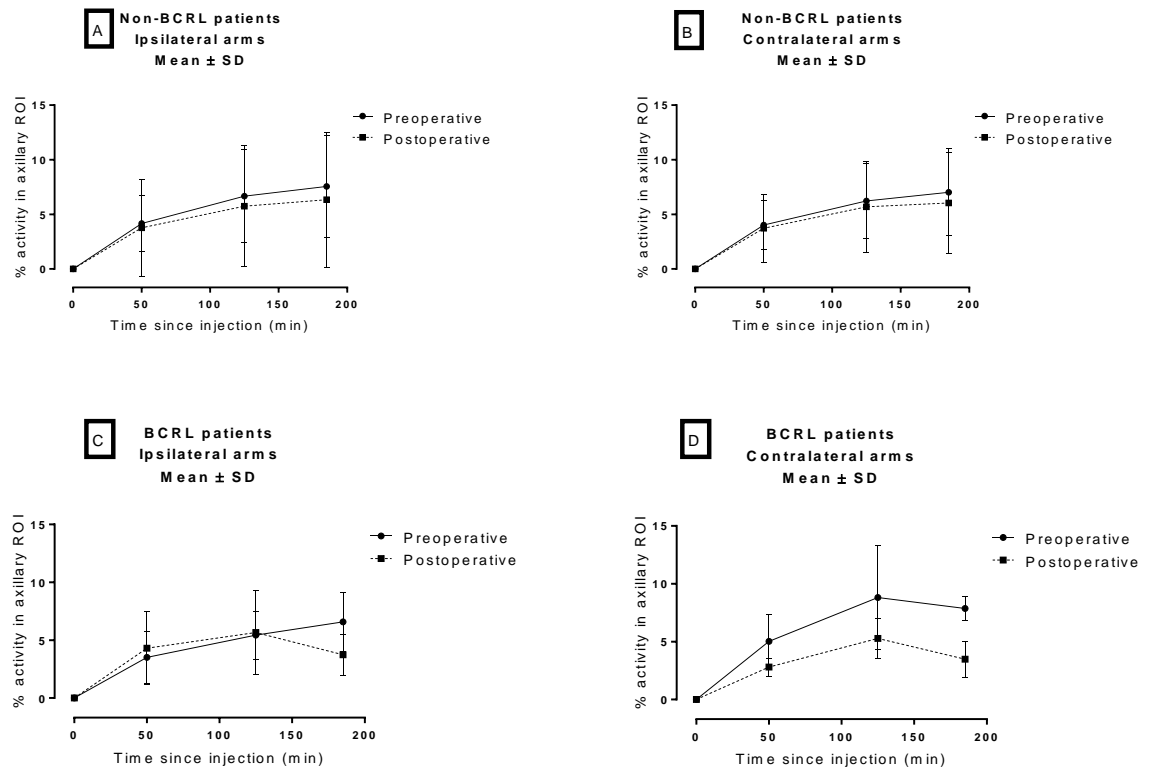


Figure 17 Axillary activity as a percentage of depot injection in the ipsilateral and contralateral upper limbs of the pre-BCRL patients ($n = 7$ pre-operatively, $n = 4$ postoperatively) and non-BCRL groups ($n = 28$ pre-operatively, $n = 4$ postoperatively).

3.8 Discussion

This study demonstrates that there is no early (i.e. after 8 weeks) effect of axillary lymph node surgery on lymph flow measured in the ipsilateral forearm muscle despite the obvious partial disruption of the axillary drainage route. There was similarly no effect on ipsilateral upper limb volume when comparing pre-operative to post-operative measurements. This indicates that BCRL is not caused directly by an acute obstruction to lymph flow at the time of surgery.

3.8.1 Incidence of BCRL

The overall incidence of BCRL in this study was 18% (7/38). It has been reported that approximately 75% of cases of BCRL occur within the first year after surgery and 90% of cases will present within three years.^{164,174,313} Although the follow-up period in this study is relatively short (58 ± 9 weeks), it is probable that 1 or 2 further patients might develop BCRL, which would make minimal difference to the overall conclusions of the study.

There is conflicting evidence in the literature regarding the association between increased incidence of BCRL and more extensive breast surgery.^{171,172 191} In this study, the extent of breast surgery did not affect the incidence of BCRL, with there being no significant difference in incidence between mastectomy and breast conserving surgery (WLE).

Several retrospective studies have suggested that lymph node positivity is related to the development of BCRL.^{160,173,174,210,314} However, the patients in those studies

had axillary radiotherapy administered if they were found to be node positive, which would have affected the prevalence of BCRL. The association between nodal positivity and the development of BCRL was examined in a recent analysis of two prospective studies of 212 patients undergoing ALND. It was observed that positive nodal status was inversely related to upper limb volume in all patients, after correcting for changes in the contralateral upper limb, raising the possibility that the inverse relationship may be due to node positive patients developing collateral lymphatic drainage prior to ANC.²¹⁴

The total number of nodes removed rather than the specific surgical procedure has been found to have a greater association with BCRL development.²¹⁰⁻²¹³ The incidence of BCRL is comparable to previous studies, affecting 33% of patients undergoing ANC and only 5% in those undergoing ANS.^{91,171,172,294}

3.8.2 Upper limb volumes

BCRL group. At the post-operative visit, three patients exhibited clinical signs of BCRL and the ipsilateral upper limb of the BCRL patients was on average 6% larger than the contralateral upper limb. This can be explained by the BCRL being early and mild and the clinical examination being more sensitive in detecting subtleties rather than an isolated statistical comparison of pre-operative and post-operative upper limb volumes. Similarly, BCRL diagnosis cannot be based simply on the comparison of ipsilateral and contralateral upper limb volumes, because there may be changes in contralateral upper limb volumes resulting from changes in body weight or structure, which would invalidate the comparison.³¹⁵ When patients were

diagnosed with BCRL, the upper limb volume was only considered relevant in the presence of additional clinical signs and symptoms of BCRL. Notably, by the follow-up visit at 58 ± 9 weeks there was no significant upper limb volume difference remaining either between upper limbs or between visits (Table 19). This can possibly be attributed to early referral of patients to the lymphoedema clinic and the commencement of compressive treatment.

Non-BCRL group. There was no significant difference in upper limb volume in patients who had not developed BCRL, either between ipsilateral and contralateral upper limbs or between pre-operative, post-operative and follow-up visits (Table 19).

3.8.3 Axillary activity

Post-operative axillary activity measurements confirmed substantial transport from the depot after surgery. This indicates that the lymphatics remained active after surgery, contrary to the lymphostasis or stopcock hypothesis. There was a general trend towards reduced axillary tracer levels, as might be expected following nodal excision, but the number of studies was too small to assess the reduction statistically. Presumably residual axillary nodes after surgery continue to drain the upper limb, which is why axillary activity is evident and why most upper limbs showed no traces of oedema at 8 weeks. Additionally, some lymph may drain into the supraclavicular nodes, which were included in the observed regions of activity. Also a lymphatic imaging study has demonstrated additional vessels post-operatively, consistent with re-routing of the lymph.³¹⁶ In lymph node positive

patients, the lymphatic system may already have started to compensate by re-routing lymph through newly developed/expanded collaterals prior to surgery – a view supported by the finding that positive lymph nodal status is inversely related to upper limb volume in patients undergoing axillary lymph node dissection.²¹⁴

3.8.4 Constitutively high fluid turnover in forearm muscle of BCRL-prone patients

Previous work has reported k in forearm muscle of 43 women without BCRL at 7 and 30 months after breast cancer surgery. A subgroup that subsequently developed BCRL had a 22% higher local muscle lymph flow (k) than non-BCRL patients. Moreover, there was a corresponding difference in the *contralateral* upper limb k values, and also in the *subcutis* k values on *both* sides.²⁹⁴ This indicated that women destined to develop BCRL experienced a greater fluid load on the lymphatic system. Chronic overload could lead to eventual lymphatic pump failure, which is a proven feature of established BCRL.²⁸⁹ Since lymph is generated from capillary filtrate, high lymph flows imply high microvascular filtration rates - the 'high filterer' hypothesis. Supporting and extending this hypothesis, the present results showed a significantly higher mean k in the pre-BCRL patients than in the non-BCRL patients. Since these patients had not yet undergone surgery, this new finding indicates a constitutional difference in fluid turnover, rather than a difference in response to surgery, a possibility not investigated in previous studies.²⁹⁴

3.8.5 Short-term effect of surgery on lymphatic drainage.

The k results showed that muscle lymph drainage was not significantly affected by axillary lymph node surgery at the 8-week time point. In this study there was a small (15%) reduction in mean $k_{\text{ipsilateral}}$ at 8 weeks post-operatively but this did not reach significance, so it is not possible to conclude that there was no reduction at all. The inherent variability in human tissue and methodology, along with the low incidence of BCRL, limits the resolution of this study. No major reduction in lymph drainage was found at 8 weeks, such as would occur if ANC had a simple stopcock effect (lymphostasis).

3.8.6 $^{99\text{m}}\text{Tc-Nanocoll}$ versus $^{99\text{m}}\text{Tc-HIG}$

One technical difference to be noted between the present study and that of Stanton *et al*, is the use of $^{99\text{m}}\text{Tc-Nanocoll}$ tracer rather than $^{99\text{m}}\text{Tc-HIG}$, which is no longer available. The $^{99\text{m}}\text{Tc-Nanocoll}$ method appears to be robust, as shown by the highly significant correlation in pre-operative k between the two upper limbs. The absolute values of k for $^{99\text{m}}\text{Tc-Nanocoll}$ were approximately half those for the smaller $^{99\text{m}}\text{Tc-HIG}$ molecule.²⁹⁴ In animal studies, the optimal particle size has been estimated at 5 nm for lymphatic drainage studies.³⁰² If smaller than this (0.05 nm – 5 nm) the particles usually diffuse into blood capillaries and therefore become unavailable to migrate through lymphatic channels.³⁰² Larger particles are prevented from entering the blood capillaries by a basement membrane and endothelial layer.^{300,302} However, if particles are larger than 100 nm, it is thought that they become trapped in the interstitial compartment for a relatively long period and show a significantly lower accumulation rate in the lymph nodes.^{296,302}

Large particles have been detected in venous blood immediately after subcutaneous injection, which is thought to be due to localised trauma from the injection site.^{296,301,302} Particle uptake by the lymphatic system is temperature dependent, enhanced by increasing temperature.³¹⁷

Binding of radiopharmaceuticals to plasma proteins is greatly influenced by:

- Charge on radiopharmaceutical molecule
- pH
- nature of protein
- concentration of anions in plasma

Protein binding affects the tissue distribution and plasma clearance of a radiopharmaceutical and its uptake by the organ/tissue of interest.³¹⁸ For proteins and particles with sizes of 1-50 nm, there could be a combination of effects affecting velocity through tissue interstitium. Reddy *et al* (2006) assessed interstitial convection. Larger molecules may be restricted to smaller number of pores (e.g. only larger pores), but they would have higher fluid velocities. However, large molecules could also interact with the extracellular matrix, with physical hindrance or charge interactions, and this would lead to a slower velocity. There could be a combination of both of these factors. Flexible and deformable chains would also move more easily through pores and be able to avoid hindrances compared with rigid shapes. Anionic molecules were also found to move faster through the interstitium than neutral molecules.³¹⁹

Nanocolloid is an aggregate of denatured human serum albumin (HSA) colloid. According to the manufacturer ~95% of particles are <80nm. However, mean size has been documented as 6.6-30 nm.^{318,320-323} The reported diameter of single HSA particles is 7 nm.³²⁴ Nanocoll particles consist of about 10 HSA particles so the molecular weight is expected to be ten times that of HSA (i.e. 10 x 67000 daltons).³²⁰ Only 30-40% of Nanocolloid in a subcutaneous depot enters the lymphatic system and a fraction of the injected dose is phagocytised by histiocytes at the injection site. Another fraction appears in the blood and accumulates mainly in the reticulo-endothelial system of the liver, spleen and bone marrow; faint traces are eliminated via the kidneys (GE Healthcare). Therefore its clearance may be further complicated and could account for the differences with ^{99m}Tc-HIG.³²⁵ Differences in the size of Nanocoll particles could lead to an underestimation of the true depot half-life. This is due to the fact that the larger sized particles may be removed faster and smaller sized particles may be limited by diffusion. In this situation the initial washout would appear monoexponential, but prolonged measurements might reveal a different trend.³²⁶ ^{99m}Tc-HIG is technetium-labelled human polyclonal immunoglobulin (HIG) and has been found to be superior to ^{99m}Tc-HSA for measuring of lymphatic function, which was attributed to its improved stability of labelling.³²⁷ HIG has a molecular weight of 150,000 daltons.³²⁸ In animal studies HIG preferentially follows the lymphatic route and has a high uptake by lymph nodes. It is able to demonstrate discrete lymph nodes and lymphatic channels.³²⁸

Mahony *et al.* (2004) aimed to find an optimal method for imaging lymphatic vessels of the upper limb and compared ^{99m}Tc -HIG and ^{99m}Tc -Nanocoll. After intradermal injection, mean removal rate constant (k) was similar for both radiopharmaceuticals and subcutaneous injection was approximately three times slower. ^{99m}Tc -Nanocoll was also found to produce only marginally inferior images than ^{99m}Tc -HIG.²⁹⁸ In contrast, Fowler *et al.* (2007) found ^{99m}Tc -HIG to be inferior to ^{99m}Tc -Nanocoll with regard to SLN identification.³²⁹ These studies confirm that ^{99m}Tc -Nanocoll is a suitable agent to use for lymphoscintigraphy.

Despite these differences ^{99m}Tc -Nanocoll generated results that again supported the higher filterer hypothesis, and moreover indicated a similar magnitude of difference (16%) as the ^{99m}Tc -HIG study (22%). In addition, it shows that lymph flow in the ipsilateral forearm muscle did not change post-surgery in either the patients who developed BCRL or in those who did not (**Figure 15**).

3.9 Conclusion

These findings indicate that axillary lymph node surgery does not significantly change local muscle lymph drainage (k) in patients with BCRL or the non-BCRL patients. The data appear to be robust as demonstrated by the high correlation coefficients between the 2 pre-operative k values. The greater mean k in pre-operative patients progressing to BCRL later was statistically significant, which is in agreement with the highly significant 22% difference reported in a previous study.²⁹⁴ In this respect the hypothesis of higher lymph flow in patients who

develop BCRL remains valid, supporting a constitutive difference between BCRL-prone and non-BCRL patients.

In conclusion, axillary lymph node surgery does not have an acute effect on local muscle lymph flow, and BCRL is not caused solely by acute, surgical obstruction of the lymphatic channels.

Subgroup	Pre-operative visit <i>n</i> = 38			Post-operative visit <i>n</i> = 33			Follow-up visit <i>n</i> = 31		
	Ipsilateral	Contralateral	<i>p</i> *	Ipsilateral	Contralateral	<i>p</i> *	Ipsilateral	Contralateral	<i>p</i> *
BCRL ^a	2846 ± 382	2805 ± 373	0.43	2943 ± 476	2780 ± 448	0.004	2861 ± 601	2726 ± 431	0.24
Non-BCRL ^b	2527 ± 760	2540 ± 729	0.66	2562 ± 773	2570 ± 732	0.69	2515 ± 795	2496 ± 760	0.45
<i>p</i> **	0.29	0.37		0.15	0.38		0.24	0.32	

Table 19 Ipsilateral and contralateral upper limb volumes (ml) measured by perometry (mean ± SD).

^a *n* = 7 at pre-operative visit, *n* = 6 at post-operative visit;

^b *n* = 33 at pre-operative visit, *n* = 27 at post-operative visit.

*Ipsilateral *versus* contralateral upper limb volumes (paired *t* test) shows a significant difference at the post-operative visit in the BCRL group (*p* = 0.004). There is no significant difference at either pre-operative or post-operative visit in the BCRL group and the non-BCRL group at any of the visits.

** BCRL *versus* non-BCRL group (unpaired *t* test) shows no significant difference between ipsilateral and contralateral upper limb volumes at any of the visits.

Subgroup	Pre-operative visit <i>n</i> = 38			Post-operative visit <i>n</i> = 33		
	Ipsilateral	Contralateral	<i>p</i> *	Ipsilateral	Contralateral	<i>p</i> *
BCRL ^a	0.0906 ± 0.0207	0.1017 ± 0.0442	0.38	0.0772 ± 0.0231	0.0828 ± 0.0033	0.59
Non-BCRL ^b	0.0857 ± 0.0217	0.0803 ± 0.0157	0.14	0.0849 ± 0.0178	0.0797 ± 0.0190	0.23
<i>p</i> **	0.58	0.25		0.47	0.43	

Table 20 Lymphatic removal rate constants *k* (%/min, mean ± SD) measured in the forearm by quantitative lymphoscintigraphy

^a *n* = 7 at pre-operative visit, *n* = 6 at post-operative visit; ^b *n* = 33 at pre-operative visit, *n* = 27 at post-operative visit

* Ipsilateral *versus* contralateral *k* (paired *t* test) shows no significant difference at either pre-operative or post-operative visit in the BCRL group or the non-BCRL group.

** BCRL vs. non-BCRL group (unpaired *t* test) shows no significant difference in *k* in the ipsilateral or contralateral upper limbs at the pre-operative and post-operative visit.

CHAPTER 4

4 Study 2: An investigation of lymphovenous communications in the upper limb in breast cancer patients

4.1 Introduction

All lymph eventually drains into the venous system, primarily via the thoracic or lymphatic duct in the neck. Studies have shown the presence of communications between peripheral veins and lymphatics, although the significance of these remains unclear, but raises the possibility of peripheral vascular drainage of lymph. It has been suggested that these peripheral lymphovenous communications (LVCs) respond to lymphatic hypertension following axillary lymph nodal surgery and provide something akin to a protective effect.^{293,330} Local vascular clearance of lymph via peripheral LVCs could in theory potentially minimise or prevent BCRL.

Previous work by this group investigated the delivery of radiolabelled autologous red blood cells to lymphatic vessels by intradermal injection. Radiolabelled-erythrocytes were injected intradermally into the hands of 4 normal subjects. Labelled erythrocytes could only access local blood through LVCs or needle trauma. Following intradermal injection, scintigraphy revealed abundant axillary activity,

indicating erythrocyte transport up upper limb lymphatics. There was no evidence of cell-bound activity in ipsilateral blood, indicating neither LVCs nor needle trauma.²⁹¹ A further study was performed in four patients 3 months after axillary surgery and intradermally injected labelled erythrocytes were recovered bilaterally in central blood in one patient. This was the only patient in this group who did not go on to develop BCRL.²⁹¹ As this study assessed only small numbers of patients post-operatively, it was difficult to know if LVCs existed prior to surgery, or simply opened up as a result of surgical intervention to the axilla. This study aims to further investigate the presence or absence of LVCs in breast cancer patients.

4.2 Study Aim

To investigate the presence or absence of lymphovenous communications (LVCs) in the upper limb in breast cancer patients, and to see if these act as a rescue mechanism in patients who do not develop BCRL.

4.3 Study Design

This study was divided into two parts: 2a and 2b. Study 2a was a prospective observational study involving patients who were recently diagnosed with unilateral breast cancer and due to undergo axillary lymph node surgery. Study 2b was designed as a retrospective observational study of patients who underwent axillary lymph node surgery for breast cancer at least three years previously; within which two groups of patients were recruited – a group with BCRL and a group without.

Patients in both studies were assessed for the presence of LVCs by injecting radiolabelled autologous RBCs into the 2nd webspace of the hand of the affected side. Images of the axilla and hand were obtained using a gamma camera and bilateral blood samples obtained from each antecubital fossa. Images were performed for qualitative lymphoscintigraphy and for calculating depot clearance from the hand. Study 2a patients (henceforth referred to as Group 1 patients) were followed up to see if they developed BCRL. Study 2b patients (Group 2 patients) were compared to firstly assess if there were any LVCs, and secondly to see if there was any difference between patients with and without BCRL.

4.4 Methods

4.4.1 Recruitment of patients

Patients studied pre-operatively (Group 1)

Six patients with recently diagnosed invasive breast cancer and due to undergo surgery, which included a level II/III ALND, at GSTT were recruited. Patients were studied in the Nuclear Medicine department. Patients attended on three occasions: pre-operatively, two to six weeks post-operatively and follow-up visits. On pre- and post-operative visits the following procedures were performed:

- (i) Clinical assessment of the upper limbs for presence of BCRL;
- (ii) Upper limb volume measurement;
- (iii) Venous pressure measurement;

(iv) Lymphoscintigraphy and venepuncture for circulating erythrocyte and plasma ^{99m}Tc concentrations (calculated as % of administered activity to assess for the presence of LVCs).

Clinical and upper limb volume assessments were performed at follow-up visits (*as detailed in Chapter 2*).

Patients studied > 3 years post-surgery (Group 2)

Ten patients with invasive breast cancer diagnosed at least three years ago and who underwent level II/III ALND as part of their surgical treatment were recruited. Two groups of patients were identified; those who had not developed lymphoedema post-operatively and those who had. Patients attended on one occasion only and underwent procedures (i)-(iv) as described above.

4.4.2 Blood sampling preparation

An intravenous cannula (18G green cannula) was inserted into a vein in the antecubital fossa of the contralateral upper limb and 5 ml blood withdrawn and radiolabelled. A three-way tap containing heparinised (2.5units/ml) saline was connected to the cannula. A second intravenous cannula (with three-way tap and heparinised saline) was inserted into a vein in the antecubital fossa of the ipsilateral upper limb for collection of blood samples.

4.4.3 Blood sample processing

For labelling with ^{99m}Tc , 1 ml heparinised whole blood was incubated for 5 min with stannous pyrophosphate (Mallinckrodt Medical, Petten, The Netherlands)

containing 0.66 µg stannous chloride and then washed with 5 ml saline. An aliquot of 0.1 ml pre-tinned packed autologous erythrocytes was incubated with 250 MBq sodium ^{99m}Tc-pertechnetate (Drytec; GE Healthcare, Bucks, UK) for 15 min and then washed twice with 5 ml saline. The ^{99m}Tc-labelled erythrocyte preparations were each reconstituted to a final haematocrit of 10% with saline. Labelling efficiency was $86.4 \pm 10.2\%$ (Appendix 4).

4.4.4 Injection site

The skin was cleaned and 0.10 ml of ^{99m}Tc-erythrocyte solution (containing approximately 20 MBq) was injected intradermally into the 2nd metacarpophalangeal joint interspace of the ipsilateral hand using a 5/8" 25G needle (0.5mm outer diameter).

Blood samples were taken as soon as injection was complete (which took two minutes), and further samples were obtained at 15, 30, 60, 120 and 180 minutes after injection. At each time point a 5 ml sample of venous blood was taken from both upper limbs.

4.4.5 Image acquisition

The scans were conducted in a temperature controlled room; ambient temperature (T_a). Skin temperature (T_{sk}) was recorded with a thermometer using thermistor probes (4600 Precision Thermometer, Measurement Specialties Inc., USA). A single camera headed camera was required for imaging, using a 256x256 matrix, low-energy high-resolution collimator. Each image took 300 sec (5 min). Images were taken at 2, 15, 30, 60, 120 and 180 minutes after injection.

At each time point two images were obtained:

- Image 1: Head one positioned above patient to include the injection site and patient's upper limb on the ipsilateral side.
- Image 2: Head one positioned above patient to include axilla on the ipsilateral side

Images were analysed using the HERMES software system.

4.5 Image analysis

4.5.1 Lymphoscintigraphy analysis

Scans were assessed for evidence of activity in the axilla after intradermal injection, to ensure accurate injection technique. In addition, lymphoscintigraphic images were reviewed for evidence of abnormal findings, especially transport through small skin lymphatics ('dermal back flow').

4.5.2 Calculation of removal rate constant (k)

As per section 3.5.1, the clearance of radiotracer from the intradermal depot was quantified using a circular region of interest (ROI) of 37.5 cm² encompassing the entire ipsilateral hand injection depot. Counts were recorded at 2, 15, 30, 60, 120 and 180min post-injection. The fraction of the counts remaining in the depot (corrected for radionuclide physical decay) was plotted semi-logarithmically against time. The slope of the linear plot of \log_e fraction *versus* time gave the fraction of

tracer removed per minute. Multiplying by 100 gave k in units of % tracer removed per min. The theory relating k to lymph flow has been discussed extensively elsewhere.^{286,288,309-311}

4.6 Blood sample analysis

4.6.1 Assessing for evidence of lymphovenous communications

After injection of 0.1 ml of ^{99m}Tc -erythrocyte, 5 ml of each of the blood samples taken was transferred into a counting tube and processed (Appendix 4). In summary, a 2 ml aliquot of each blood sample was diluted with 8 ml saline and centrifuged (500 g , 5 min) to wash away free ^{99m}Tc -pertechnetate released by the erythrocytes. The supernatant was discarded and the remaining ~1 ml of packed erythrocytes (i.e. from the 2 ml whole blood sample) was suspended in 8 ml saline. A 1 ml aliquot of this suspension was transferred to a counting tube and the remaining 8 ml suspension was centrifuged (500 g , 5 min). Finally, 1 ml aliquot of the supernatant was taken and all tubes were assayed in a gamma counter (Wallac Wizard, Perkin Elmer, UK).

A difference in the counts/ml in the cell suspension (^{99m}Tc -erythrocytes plus free ^{99m}Tc) and counts/ml $\times (1 - 0.11)$ in the supernatant (free ^{99m}Tc) that was deemed significant (see below) was interpreted as evidence of intact ^{99m}Tc -labelled erythrocytes in the circulation. In such cases, this difference was considered to represent shunted labelled erythrocytes and expressed as % of injected activity per

litre of blood. Blood volume was estimated from weight and height³³¹ and applied to contralateral samples only to calculate the % of injected labelled cells in the general circulation.

Blood volume = Red cell mass + plasma volume

Red cell mass (ml) = 822 x Surface Area

Plasma volume (ml) = 1395 x Surface Area

Surface area (m²) = weight(kg)^{0.425} x height(cm)^{0.725} x 0.007184

Excess activity in ipsilateral samples, over and above the activity in contralateral samples, that would be consistent with shunting between the ipsilateral antecubital fossa and injection site (see Statistical analysis section 4.7), was noted but not quantified.

4.6.2 Correcting for activity remaining in depot in patients with LVCs.

Residual activity in the hand injection depot was calculated from the counts in the ROI from the lymphoscintigraphic images. From the depot residual activity the quantity of erythrocytes that had left the depot was calculated. The quantity that had arrived in the circulation, calculated from the contralateral sample (see previous section) was expressed as a fraction of the quantity that had left the depot. This served as a measure of the extent of lymphovenous shunting.

4.7 Statistical analysis

Results are shown as the mean \pm standard deviation (SD). Linear regression analysis was used to analyse the slope of the semi-logarithmic depot clearance plot, which was assumed to be mono-exponential. Group comparisons were made using Student's unpaired *t* test. Fisher's exact test was used for categorical data due to the small sample sizes. Analysis was performed using GraphPad Prism (version 6; San Diego, CA, USA). A *p* value of ≤ 0.05 was accepted as significant. To calculate the difference between cell suspension (WB) and supernatant (SN) counts, the SD of the total counts was calculated as $(\text{WB counts} + \text{SN counts})^{0.5}$. A difference in counts between cell suspension and supernatant of >2 SDs was considered significant and to indicate lymphovenous communication.

4.8 Results

4.8.1 Patient data

Group 1

Pre-operative measurements were performed in six women. No images or blood samples were obtained for one of these patients and she was therefore excluded from further analysis. The remaining five patients attended both the post-operative study and the follow-up visit. The mean age of patients at the time of surgery was 54 ± 3 years (range: 48 - 56 years) and the body mass index (BMI) was $23.6 \pm 2.3 \text{ kg/m}^2$ (Table 21). The intervals between surgery and subsequent assessments were 9 ± 6 weeks (post-operative visit) and 19 ± 5 months (follow-up

visit). None of the patients developed BCRL. All five patients were right hand-dominant and a total of 3/5 (60%) of patients had surgery to their dominant side.

	(<i>n</i> = 5)
Age (years)	53.8 ± 3.2
BMI (kg/m ²)	23.6 ± 2.3
Ipsilateral arm volume (ml)	2242 ± 260
Nodes removed	15.8 ± 7.9
Positive nodes	0.4 ± 0.6

Table 21 Group 1 patients' clinical details (mean ± SD)

Group 2

We recruited ten patients of whom seven patients had BCRL and three patients did not. All patients were at least three years after axillary lymph node dissection for breast cancer. All patients had previously undergone axillary node clearance surgery (ANC) with an average of 21.4 ± 4.6 nodes removed, of which 6.4 ± 10.1 nodes were found to be positive. Comparing the BCRL and non-BCRL patient groups revealed a statistical difference in the number of nodes removed, with more nodes removed in patients in the BCRL group (unpaired *t* test $p = 0.04$) although the number of positive nodes remained insignificant ($p = 0.96$). The BMI difference approached significance with a higher BMI in patients in the BCRL group compared with the non-BCRL group ($p = 0.05$). The other significant difference was the ipsilateral upper limb volume, which was significantly larger in the BCRL group when compared with the non-BCRL group (unpaired *t* test, $p = 0.015$) (Table 22).

	BCRL (<i>n</i> = 7)	Non-BCRL (<i>n</i> = 3)	<i>p</i> *
Age (years)	50.2 ± 9.2	46.7 ± 9.0	0.58
BMI (kg/m ²)	30.4 ± 3.1	25.7 ± 1.8	0.05
Nodes removed	21.2 ± 4.5	14.3 ± 1.5	0.04
Positive nodes	4.7 ± 9.5	4.0 ± 4.0	0.96
Ipsilateral arm volume (ml)	3155 ± 426	2220 ± 481	0.02

Table 22 Group 2 patients' clinical details: Comparison between BCRL and non-BCRL patients (mean ± SD)

**unpaired t test.*

4.8.2 Intradermal lymph drainage (*k*)

^{99m}Tc-labelled erythrocyte clearance from the ipsilateral hand injection depot was evident from the earliest time points. The fraction of the counts remaining in the depot (corrected for radionuclide decay) was plotted semi-logarithmically (Figure 18). To assess the proportion of variability of measurement of *k* in our data set, the results from patients in both Group 1 and 2 were pooled. The median (interquartile) correlation coefficient of the exponential fit to the data points (*n* = 18) was 0.97 (0.95-0.99). Activity rapidly reached the axilla (within 15 min) in all patients. In all seven patients with BCRL in Group 2, prominent activity was seen in the dermal lymphatics throughout the affected arm indicating that the injected ^{99m}Tc-labelled erythrocytes had indeed entered the lymphatic system (Figure 19).

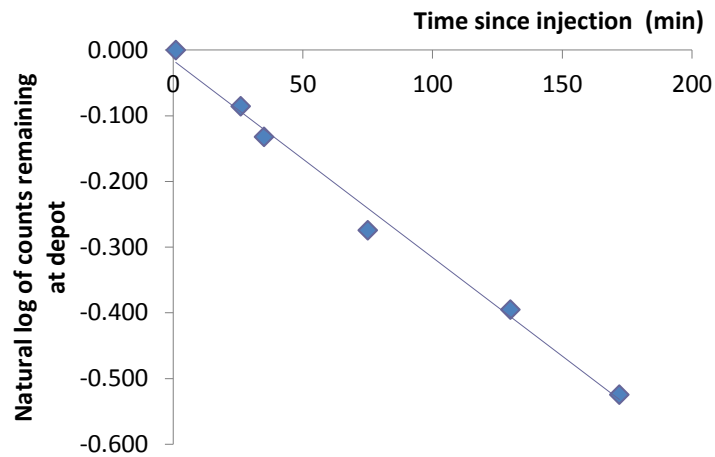


Figure 18 Natural log of counts remaining in the intradermal injection depot of ^{99m}Tc -erythrocytes

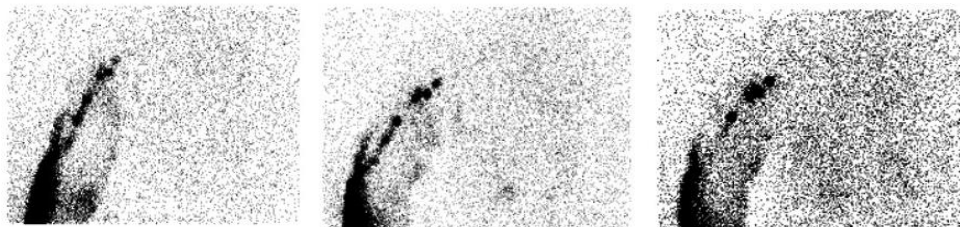


Figure 19 Images of the axilla 15, 30 and 180 min after intradermal injection of ^{99m}Tc -RBC showing activity in dermal lymphatic vessels.

4.8.3 Quantitative studies

Depot clearance rates

There was no significant change in k between pre- and post-operative studies in Group 1 patients, with corresponding mean values of 0.25 ± 0.06 and 0.26 ± 0.10 %/min, respectively.

When the pre- and post-operative k values in Group 1 were pooled, the mean value tended to be lower than the mean k value in the 7 patients with established BCRL in

Group 2, with respective values of 0.28 ± 0.11 and 0.39 ± 0.12 %/min, although the difference was of marginal significance ($p = 0.087$). The mean k for non-BCRL patients in Group 2 was 0.36 ± 0.26 %/min.

Shunting through LVCs

Group 1 contralateral evidence: Contralateral blood samples were obtained from Group 1 patients in 4 pre-operatively and 4 post-operatively. No shunting could be detected in 2/4 pre-operative studies and 2/4 post-operative studies. However in 4 studies, shunting was detected with maximum values of 1.6% and 1.8% (pre-operatively and post-operatively, respectively, in the same patient) and 6.6% and 2.3% (pre-operatively and post-operatively in separate patients, respectively) of activity that had left the depot.

Group 2 contralateral evidence: Contralateral blood samples were obtained from 7 patients in Group 2. No shunting could be detected in 4 of them, including all 3 patients without BCRL. However, shunting was detected in 3 patients, all with BCRL, with maximum values of 0.1%, 0.7% and 0.5% of activity that had left the depot. In subjects showing contralateral evidence of shunts, the labelled red cells accumulated progressively in the contralateral samples over time, indicating that the shunt is continuous in its operation. (Figure 20)

Ipsilateral samples were obtained in 5 Group 1 studies and 7 Group 2 studies. Ipsilateral shunting located between the depot site and antecubital fossa was

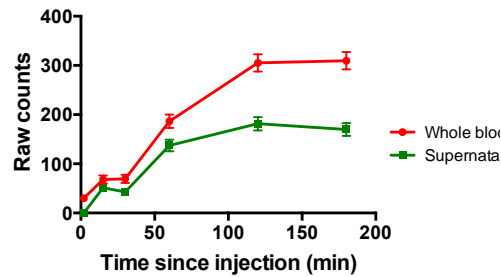
inferred when erythrocyte-associated activity was higher in ipsilateral than simultaneous contralateral samples.

Group 1 ipsilateral evidence: Excess ipsilateral activity was seen in only one study, in the 15 min sample of a patient who did not have contralateral shunting. There was no ipsilateral excess in the other 4 studies, in 2 of which there was evidence of shunting in the contralateral samples.

Group 2 ipsilateral evidence: Ipsilateral counts indicating shunting in 2 subjects of group 2; unfortunately no contralateral samples were available in either subject. In 2 patients with shunting based on contralateral samples, no excess ipsilateral activity was detected. So, in general, the criterion of ipsilateral excess counts did not provide convincing evidence of shunting in the distal ipsilateral arm.

Comparison of removal rate constant and shunting

There was no difference in k values between patients with and without evidence of LVC shunting in the contralateral blood, with corresponding values of 0.36 ± 0.14 ($n = 6$) and 0.31 ± 0.17 %/min ($n = 7$), respectively ($p = 0.57$).



A

B

Figure 20 Counts in blood samples from the contralateral limb. **A;** The increase in counts in a Group 2 patient with BCRL indicated evidence of LVCs. **B;** There was no evidence of LVCs in a Group 2 patient without BCRL in which there was no significant difference in counts. Vertical bars are the SDs of individual counts.

4.9 Discussion

Study 2a was designed to study patients in a prospective manner, but there was considerable difficulty in patient recruitment. Patients found the study to be both time-intensive and invasive. Venepuncture of the ipsilateral upper limb soon after axillary lymph node surgery also caused some concern for patients regarding the perceived risk of the development of BCRL. Several patients were also undergoing chemotherapy peri-operatively, which made venous access for the study more difficult. In addition, due to the preliminary nature of this study and after assessing initial results, it was difficult to predict how long it would take for potential LVCs to develop or present themselves after surgery. The decision was made to suspend Study 2a after recruiting six patients and to commence Study 2b; the planned retrospective study. Study 2b was less time-intensive for patients and as it only involved one visit. Furthermore, those who had not developed BCRL were less

apprehensive about venepuncture as they were at least 3 years after axillary lymph node surgery.

The ability to deliver intact red cells to lymphatic vessels is itself of great interest, especially in the context of the general intra-lymphatic administration of particulate materials, liposomes and intact blood cells. Moreover the rate of removal of the red cells by lymphatic transport was similar to the rate of removal of intradermal human immunoglobulin (0.24 - 0.47%/min), in keeping with unimpeded transport by bulk flow along the lymphatics.³³²

This results from this study showed evidence of shunting of labelled erythrocytes from the interstitial depot in the hand into the blood circulation in a subsection of subjects. Since erythrocytes are presumably unable to pass through the axillary lymph nodes to reach the bloodstream, their passage appears to indicate the presence of LVCs more distally in the arm. Needle trauma was considered as a potential artefactual source of activity from depot into the circulation. However, trauma would be expected to result in (i) a rapid, unsustained appearance of labelled cells in blood, unlike the slow, continuous accumulation evident in Figure 20, and (ii) a clear ipsilateral excess over contralateral counts. Although rapid ipsilateral appearance of labelled cells was observed in one patient, the contralateral samples provided no evidence of shunting in that patient. The likeliest, though unproved, location of lymphovenous shunting is therefore the upper arm or axilla. With respect to the axilla, this may be the post-operative consequence of

axillary lymph node resection followed by regeneration of lymphatic vessels. Alternatively, in order to explain pre-operative shunting, lymph vessel-to-lymph vessel shunting may take place so that the labelled erythrocytes bypass lymph nodes.

Shunting, as indicated by contralateral cell-bound counts, was much more marked in terms of the percentage of shunting in Group 1 patients, who on follow-up did not show any clinical evidence of BCRL. On the other hand, the 3 patients in Group 2 who did not develop BCRL, displayed no evidence of shunting from contralateral sampling, whilst of the 3 with evidence of a very small degree of shunting, 3 had BCRL.

4.10 Conclusion

The findings make it difficult to conclude that LVCs pre-exist constitutionally and/or develop in response to axillary surgery. Furthermore, the patient numbers are insufficient to determine whether, or the extent to which, LVCs protect against BCRL. Since the size of the shunt was typically less than 1-2% of the lymph flow, it seems unlikely that LVCs are a major factor influencing the likelihood of BCRL development. Given the paucity of explanations for the development of BCRL in only a minority of breast cancer patients undergoing broadly similar treatment to the axilla, further studies into LVCs are justified.

CHAPTER 5

5 Study 3: An investigation into a constitutional ‘global’ lymphatic dysfunction in patients with BCRL

5.1 Introduction

There have been studies into BCRL that have yielded observations indicating that some breast cancer patients may be more prone to developing BCRL than others who have had similar treatment. Abnormalities have also been found in the lymphatic vessels of the contralateral, non-swollen upper limbs of patients with BCRL in addition to the ipsilateral swollen upper limb. These findings point to a constitutional predisposition to BCRL.

A significant drawback of studying patients with established BCRL is that compensatory changes may take place in the contralateral upper limb if the patient is inclined to preferentially use this limb. Changes that may take place in the contralateral upper limb would preclude any opportunity to study constitutional factors. If there is a constitutional lymphatic disturbance that predisposes patients to BCRL, it would be reasonable to hypothesise that there is a global impairment of lymphatic function and that consequently it may be possible to detect lymphatic abnormalities in lower limbs.

5.2 Study Aim

To investigate if there is a functional disturbance in the lymphatics of the lower limbs in breast cancer patients with BCRL compared with those without BCRL.

5.3 Study Design

This is a prospective, non-randomised, multicentre study of a cohort of women diagnosed with breast cancer. All patients were studied using quantitative lymphoscintigraphy of the lower limbs. None of the patients had any known lower limb pathology.

5.4 Methods

5.4.1 Recruitment of patients

Thirty patients with invasive breast cancer diagnosed at least three years previously and who underwent level II or III ALND as part of their surgical treatment were recruited. Two groups of fifteen patients were identified: those who had developed BCRL post-operatively and those who had not. Patients attended on one occasion and the following procedures were performed:

- (i) Clinical assessment for the presence of BCRL in upper and lower limbs
- (ii) Upper limb volume measurement
- (iii) Lymphoscintigraphy

5.4.2 Lymphoscintigraphy: injection technique and image acquisition

Differing techniques for lower limb lymphoscintigraphy include intradermal injection compared with subcutaneous injection, varying amounts of limb exercise

of the lower limb in between imaging, timing of images and using different radiopharmaceuticals.³¹⁷ For the patients in this study, subcutaneous injection was used as per British Nuclear Medicine Society Guidelines. Lymphoscintigraphy was conducted in a temperature-controlled room. With the participant positioned supine, aliquots of 0.1 ml of ^{99m}Tc-Nanocoll solution (each containing 20 MBq) were injected subcutaneously into the first web-spaces of both feet using a 25 gauge needle (outer diameter 0.51mm, Terumo, Belgium). To confirm that blood vessels were not penetrated, the syringe was aspirated prior to injection. With the participant lying supine, gamma camera images of the full body from chest downwards were obtained at 5, 45 and 150 min after injection. The injection depot was also imaged in order to calculate depot clearance rate (*k*) (Figure 21).

Dedicated static images were performed for quantification of ilio-inguinal nodal activity at 45 and 150 min post-injection. This involved placement of a known quantity of radioactivity (a 'standard') on the thigh within the field of view of the camera (Figure 22). Whole body images took approximately 17 min to acquire, while depot and quantification images took 5 min each.



Figure 21 Gamma camera positioning for depot image



Figure 22 Gamma camera positioning for quantification image

5.5 Image analysis

All scans were reviewed independently by two observers who have extensive experience in lymphoscintigraphy, and who were blinded to the clinical details of patients. Lymphoscintigraphy was classified as normal or abnormal using conventional criteria as outlined below. Scans were deemed abnormal if they showed evidence of delay in lymph flow to inguinal nodes or abnormalities related to lymph diversion in either lower limb (Table 23).

5.5.1 Calculation of the removal rate constant (k)

As per section 3.5.1, the clearance of radiotracer from the interstitial depot was quantified using a circular region of interest (ROI) of 37.5cm^2 encompassing the entire foot injection depot. Counts were recorded at 5, 45 and 150 min. The fraction of the counts remaining in the depot (corrected for radionuclide physical decay) was plotted semi-logarithmically against time. The slope of the linear plot of \log_e fraction *versus* time gave the fraction of tracer removed per minute. Multiplying by 100 gave k in units of % tracer removed per min. This equals the local lymph flow (ml/min) per 100ml of interstitial fluid in which the tracer is distributed.

Abnormalities related to delay	<ul style="list-style-type: none"> • <i>No activity in ilio-inguinal nodes by 45 min</i> • <i>Obviously reduced activity in ilio-inguinal nodes by 150 min</i> • <i>Asymmetry in ilio-inguinal nodes at 150 min (>50%)</i>
Abnormalities related to lymph diversion	<ul style="list-style-type: none"> • <i>Dermal backflow</i> • <i>Popliteal node visualisation</i>

Table 23 Criteria for abnormal lymphoscintigraphy

5.5.2 Quantification analysis

The percentage of injected radioactivity accumulating in the ilio-inguinal nodes was calculated at the 45 and 150 min time-points. This involved the placement of the 'standard' in close proximity to the ilio-inguinal nodes and within the field of view of the camera. The same sized ROIs were drawn over the anterior images at both

time-points, encompassing (1) the 'standard' (2) right ilio-inguinal nodes and (3) left ilio-inguinal nodes. Counts were corrected for background and decay.

5.6 Control group

A retrospective control patient group was identified and images analysed by the same method as described above. This group comprised 24 female patients, all of whom were referred for lower leg lymphoscintigraphy for a range of clinical indications. None of the patients demonstrated clinical evidence of lower limb lymphoedema. These patients underwent lymphoscintigraphy with the aim of confirming normal lymphatic function. Quantification was also recorded as normal in this group. Clinical details and final diagnoses for these patients are shown in Table 24.

5.7 Statistical analysis

Results are shown as the mean \pm standard deviation. Linear regression analysis was used to analyse the slope of the semi-logarithmic depot clearance plot, which was assumed to be mono-exponential. Group comparisons were made using Student's unpaired *t* test. Fisher's exact test was used for categorical data due to the small sample sizes. Analysis was performed using GraphPad Prism (version 6; San Diego, CA, USA). A *p* value of ≤ 0.05 was deemed to be statistically significant.

Presentation	Other clinical information	Final diagnosis
Bilateral leg swelling	Family history of leg swelling	Normal
Bilateral leg swelling		Lipoedema
Bilateral knee effusions	Arthritis, obesity	Rheumatic swelling
Bilateral leg swelling	Family history of 'big legs'	Lipoedema
Bilateral leg swelling		Lipoedema
Bilateral leg swelling	Hamartoma of left leg	Normal
Bilateral leg swelling	Short stature	Normal
Bilateral leg swelling	Dercum's disease	Lipoedema
Bilateral leg swelling	Prada Willi syndrome	Obesity
Bilateral leg swelling	Family history of lipoedema, obese	Lipoedema
Bilateral leg swelling		Normal
Bilateral leg swelling	Family history of lipedema, anorexia	Lipoedema
Bilateral leg swelling		Normal
Bilateral leg swelling	Lipodermatosclerosis	Lipoedema
Minor transient leg swelling	Leg swelling after flights	Normal
Painful legs		Dercum's disease
Bilateral leg swelling		Lipoedema
Bilateral leg swelling		Lipoedema
Left arm swelling	Breast implants	Normal
Bilateral leg swelling		Lipoedema
History of left foot swelling	Previous cellulitis	Cellulitis
Bilateral leg swelling	Panniculitis, small varicosities	Erythema nodosum
Bilateral leg swelling		Normal
Bilateral leg swelling	Family history of lipedema	Lipoedema

Table 24 Clinical details of control group. All patients had normal lymphoscintigraphy and no clinical evidence of lymphoedema

5.8 Results

5.8.1 Patient data

In all, in addition to the control group, 30 patients were recruited into this study of whom 15 patients had BCRL and 15 patients did not. Patients in the non-BCRL group were at least three years after having undergone axillary lymph node dissection for breast cancer. None of these patients demonstrated any obvious clinical abnormality of the lower limbs. Clinical, surgical and pathological details for patients are summarised in Table 25. The average time for onset of BCRL after surgery was 12.5 ± 17.4 months (range 1-48 months). Thirteen of 15 patients (87%) developed BCRL within a year of surgery. All patients underwent axillary node clearance surgery (ANC) with an average of 14.9 ± 6.0 nodes removed, of which 3.0 ± 6.4 nodes were found to be positive. A significant number of patients had systemic therapy for their breast cancer, either in the form of endocrine therapy (80%) or chemotherapy (90%). There was only one patient who did not have any form of systemic therapy. Comparing the BCRL and non-BCRL patient groups revealed no statistical differences in patient factors (Table 26). The patients in each group were well matched. The only significant difference was the ipsilateral upper limb volume, which was significantly larger in the BCRL group than the non-BCRL group (unpaired *t* test, $p = 0.005$).

Patient ID	Age (yrs)	Breast surgery	Axillary surgery	Number of lymph nodes removed (+)		Histology			
						Grade	Type	Size (mm)	ER status
100B*	59	WLE	ANC	14	(12)	2	IDC	25	+
101B*	56	WLE	ANC	4	(0)	3	IDC	6	-
102B*	55	Mx	ANC	13	(1)	3	IDC	25,14	-
103B*	63	Mx	ANC	33	(33)	2	IDC	42	+
104B*	59	WLE	ANC	12	(3)	2	IDC	22	+
105B*	62	Mx	ANC	18	(2)	2	IDC& ILC	35	+
100G*	58	WLE	ANC	13	(0)	2	IDC	28	-
101G	46	Mx	ANC	12	(1)	3	IDC	50	-
102G*	49	WLE	ANC	16	(2)	2	IDC	20	+
103G	53	WLE	ANC	16	(1)	1	IDC	20	+
104G	66	Mx	ANC	21	(1)	2	IDC& ILC	11,11	+
105G	38	Mx	ANC	22	(0)	2	ILC	15	+
106G*	52	WLE	ANC	16	(2)	2	ILC	32	+
107G*	53	WLE	ANC	19	(0)	3	IDC	22	+
108G*	39	WLE	ANC	17	(0)	3	IDC	12,17	+
109G*	66	WLE	ANC	17	(1)	2	IDC	20	+
110G*	41	WLE	ANC	8	(1)	3	IDC	57	+
111G*	48	Mx	ANC	14	(1)	2	IDC	47	+
112G	43	Mx	ANC	18	(6)	3	IDC	80	+
113G	40	WLE	ANC	7	(0)	2	MUC	50	+
114G	48	Mx	ANC	10	(5)	2	IDC	36,8	+
115G*	61	Mx	ANC	22	(1)	2	MIC	57	+
116G	46	WLE	ANC	13	(5)	3	IDC	32	+
117G	60	WLE	ANC	14	(0)	2	IDC	28	-
118G	52	Mx	ANC	6	(0)	2	IDC	21	+
119G	67	WLE	ANC	14	(1)	1	TUB	10.5	+
120G	51	WLE	ANC	13	(3)	2	IDC	6,2,2,2	+
121G	44	Mx	ANC	3	(0)	3	IDC	50	-
122G	61	WLE	ANC	19	(2)	2	IDC	17	+
123G	68	Mx	ANC	22	(11)	1	CRI	16,6	+

Table 25 Clinical, surgical and histopathological details of patients

*patients with BCRL; ANC, axillary clearance surgery; ER, oestrogen receptor; WLE, wide local excision; Mx, mastectomy; IDC, invasive ductal carcinoma no special type; ILC, invasive lobular carcinoma; MUC, mucinous carcinoma; MIC, micropapillary carcinoma; TUB, tubular carcinoma; CRI, cribriform type carcinoma.

	BCRL (<i>n</i> = 15)	Non-BCRL (<i>n</i> = 15)	<i>p</i>
Age (years)	55.0 ± 7.2	54.8 ± 7.8	0.44
Body mass index (kg/m ²)	29.9 ± 4.7	27.8 ± 4.8	0.25
Ipsilateral arm volume (ml)	3231 ± 774	2561 ± 357	0.01
Nodes removed	15.7 ± 6.5	14.7 ± 5.6	0.48
Positive nodes	3.9 ± 8.6	2.4 ± 3.1	0.49
Endocrine therapy	12 (80%)	12 (80%)	1
Chemotherapy	14 (93%)	13 (80%)	1
Chemotherapy (taxane-based)	7 (47%)	6 (40%)	1
No systemic therapy	0	1	1
Abnormal scans	10 (67%)	7 (47%)	0.46

Table 26 Comparison between BCRL and non-BCRL groups

5.8.2 Image analysis

In this study, none of the control group patients (*n* = 24) demonstrated clinical evidence of lymphoedema in their lower limbs. The scans all confirmed normal lymphatic function bilaterally, with no evidence of delay in lymph transit or diversion of flow. In complete contrast, 17/30 breast cancer patients were found to have abnormal lower limb lymphoscintigraphy compared with 0/24 in the control group, which was highly significant (*p* < 0.0001, Fisher's exact test). Despite this finding, there was no difference in the number of abnormal scans in the BCRL group compared with the non-BCRL group, with 10/15 and 7/15 abnormal scans respectively (*p* = 0.46, Fisher's exact test).

Table 27 categorises scans based on whether they were normal or abnormal. Unpaired *t* test showed no obvious differences in the patient factors of either group, including difference in BMI (*p* = 0.77), number of nodes removed (*p* = 0.14), endocrine therapy (*p* = 0.67) or chemotherapy (*p* = 0.56).

	Normal scans (<i>n</i> = 13)	Abnormal scans (<i>n</i> = 17)	<i>p</i>
Age (years)	54.3 ± 9.4	52.9 ± 8.7	0.66
Body mass index (kg/m ²)	28.6 ± 3.6	29.1 ± 5.7	0.77
Nodes removed	13.4 ± 5.1	16.9 ± 7.0	0.14
Positive nodes	2.4 ± 3.7	4.0 ± 8.9	0.55
Endocrine therapy	11 (85%)	13 (76%)	0.67
Chemotherapy	11 (85%)	16 (94%)	0.56
Chemotherapy (taxane-based)	5 (38%)	8 (47%)	0.72
No systemic therapy	0	1	1
Patients with BCRL	5 (38%)	10 (59%)	0.46

Table 27 Patients grouped according to whether images were normal or abnormal

The image analysis for the abnormal scans (Table 28) details the individual findings for these patients.

Patient ID	Image analysis findings
100B*	Popliteal node visualisation L side
101B*	No activity 45 min R side
102B*	No activity 45 min bilaterally
105B*	No activity 45 min R side
100G*	Asymmetry at 150 min
102G*	Asymmetry at 150 min
104G	No activity 45 min bilaterally and popliteal node visualisation L side
106G*	No activity 45 min bilaterally
108G*	Popliteal node visualisation bilaterally
110G*	Asymmetry at 150min
111G*	No activity 45 min R side
113G	Asymmetry at 150 min
114G	No activity 45 min bilaterally and asymmetry at 150 min
116G	Popliteal node visualisation R side
117G	Popliteal node visualisation R side
118G	No activity 45 min bilaterally
123G	Popliteal node visualisation L side

Table 28 Abnormal image findings in BCRL and non-BCRL patients

*patients with BCRL; L, left, R, right

Six of 17 patients demonstrated popliteal node visualisation indicating lymphatic diversion (Figure 23). None of the patients demonstrated dermal backflow. Twelve

of 17 patients showed either no activity at 45 min or asymmetry at 150 min, which are both categorised as abnormalities related to delay in lymph flow (**Figure 24** and Figure 25).

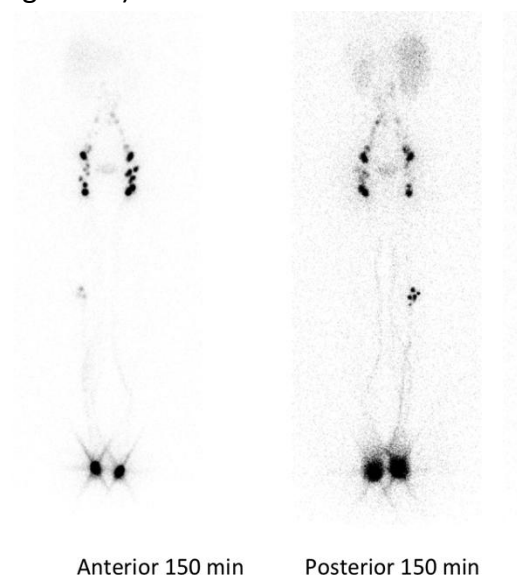


Figure 23 Images of the lower limbs, including foot depots. Popliteal node activity, signifying lymph diversion, is evident in the right lower limb. Popliteal nodes are seen most clearly on posterior images. This image also shows asymmetry in the ilio-inguinal lymph node activity.

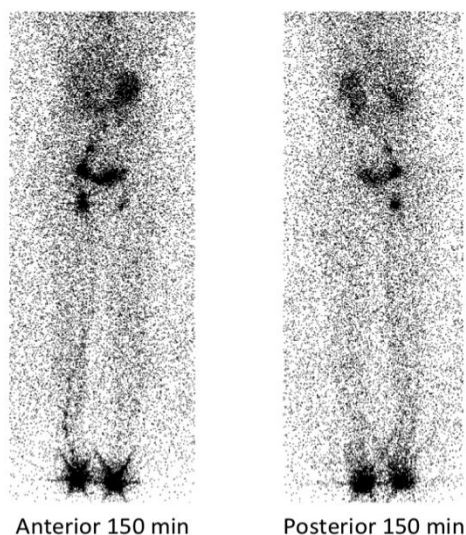


Figure 24 Images of the lower limbs. There is asymmetry of the activity in the ilio-inguinal nodes at 150 minutes, with decreased activity in the ilio-inguinal nodes of the left lower limb.

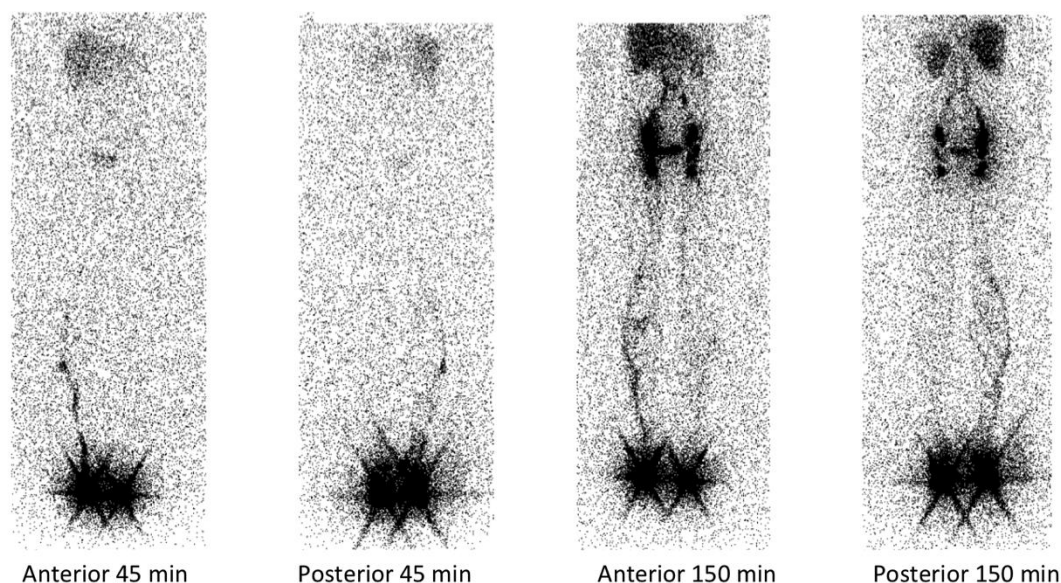


Figure 25 Images of the lower limbs. There is no activity in ilio-inguinal nodes at 45 minutes. The 150 min scan of the same patient also shows asymmetry in the ilio-inguinal nodes.

5.8.3 Lymph flow (k)

The rate of ^{99m}Tc -Nanocoll elimination from each foot injection depot was calculated for all 30 patients. However, there are only 14 sets of data available for analysis. The remaining 16 sets of data were not used because the time-related changes in decay-corrected count values were not interpretable. The counts were recorded at three time-points, but in several cases the number of counts (once corrected for decay) actually increased over time. This led to very low correlation coefficients for the exponential fit. For all imaging time-points, the distance of the patient from the camera was kept as constant as possible. To ensure that inconsistency in counts was not due to the patient being placed at a slightly different distance from the camera, the sensitivity of the camera was tested. A standard containing 25 MBq of ^{99m}Tc -Nanocoll was placed at varying distances (in

2.5cm increments) from the camera with each image taking 1 minute. The counts were recorded using a ROI over the activity and corrected for decay (Table 29). The results showed that although the number of counts and sensitivity of the camera appeared to decrease, the difference caused by the varying the distance between the source of radioactivity and the camera was not clinically significant (Figure 26).

Distance of radioactive source from camera head (cm)	Counts (corrected for decay)	Sensitivity of the camera (counts per second per MBq)
5	121662	86.7
7.5	120829	86.1
10	120777	86.0
12.5	119673	85.2
15	119826	85.3
17.5	118949	84.7
20	117583	83.7

Table 29 Measurement of corrected counts of ^{99m}Tc -Nanocoll at varying distances from the camera head and corresponding sensitivity of the camera (in counts per second per MBq)

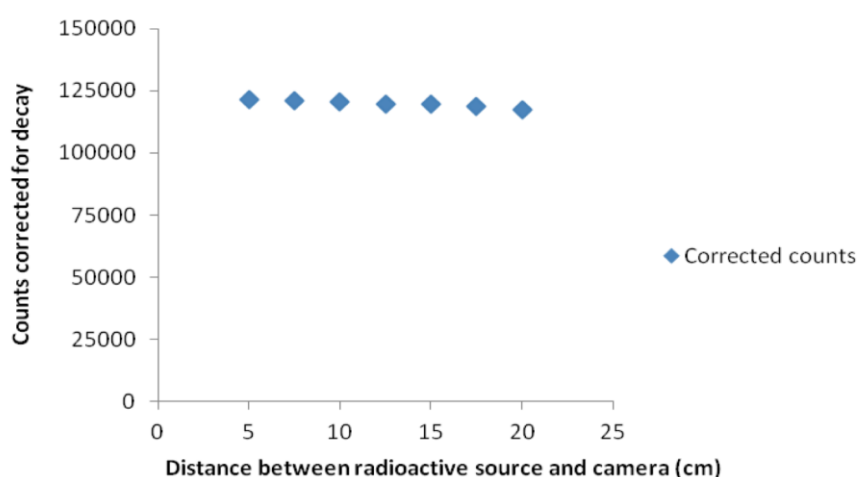


Figure 26 Plot of corrected counts for radioactive source at varying distance from the camera head (cm)

The results for those patients included in the analysis are shown in Table 30 and Table 31. Mean k for the BCRL group ($n = 8$) was 0.12 ± 0.06 %/min and 0.14 ± 0.09 %/min for the non-BCRL group ($n = 6$) ($p = 0.58$, unpaired t test) (Table 32). When separating patients according to whether they had normal ($n = 6$) or abnormal scans ($n = 8$), k was 0.12 ± 0.05 %/min and 0.14 ± 0.08 %/min respectively ($p = 0.36$, unpaired t test) (Table 33 k (%/min) values for both limbs when comparing normal and abnormal scans (mean \pm SD). The results for these individual patients are shown in tables Table 34 and Table 35. Some patients had unilaterally abnormal scans ($n = 7$) with the contralateral limb appearing normal on lymphoscintigraphy. To assess if k was significantly different depending on whether lymphoscintigraphy was normal or not, k for normal limbs (0.13 ± 0.08 %/min) was compared with the abnormal limbs (0.13 ± 0.06 %/min). This was not significant ($p = 0.98$, unpaired t test).

5.8.4 Quantification of ilio-inguinal nodal activity

Quantification was calculated for 22/30 patients, 8 in the BCRL group and 14 in the non-BCRL group. The failure of quantification in the remaining 8 patients was a technical problem with inaccurate image acquisition, rather than patient factors. The results are shown in Table 36 & Table 37. At 45 minutes the mean ilio-inguinal nodal activity, calculated as a percentage of activity of the depot injection, was not significantly different in the lower limbs of patients with BCRL compared with non-BCRL patients ($0.75 \pm 1.4\%$ vs. $0.84 \pm 1.2\%$; $p = 0.82$). However, at 150 minutes, the activity was found to be significantly lower in the lower limbs of patients with BCRL compared with non-BCRL patients. When the quantification was calculated for

individual lower limbs of the BCRL and non-BCRL patients, the mean ilio-inguinal nodal activity was $2.7 \pm 2.5\%$ and $5.9 \pm 4.8\%$ respectively, $p = 0.006$ (Table 38). When the quantification was averaged for each patient, rather than considering each limb individually, the difference in mean ilio-inguinal nodal activity between the BCRL and non-BCRL patients remained significant ($2.4 \pm 1.9\%$ vs. $5.9 \pm 4.2\%$, $p = 0.026$). This provides objective evidence of abnormal lymphatic function in the lower limbs of patients with BCRL compared to those without BCRL.

5.9 Discussion

The aim of this study was to explore the possibility that patients who develop upper limb BCRL have a constitutional global lymphatic abnormality that may be detectable in their lower limbs. This study has demonstrated that patients with upper limb BCRL have reduced lower limb lymphatic function as evidenced by lower ilio-inguinal quantification when compared with non-BCRL patients. An additional important observation was that a large percentage of breast cancer patients had abnormal lower limb lymphatic function irrespective of whether they had upper limb BCRL or not and this was observed in the absence of any lower limb clinical abnormalities. This was in sharp contrast to patients in the control group all of whom had completely normal scans.

The criteria we used to establish which patients had normal or abnormal scans were based on other studies that have used similar injection techniques and radiotracers.^{295,333-335} Lymphoscintigraphy imaging is a sensitive method of objectively differentiating between abnormal limb swelling due to lymphatic

pathology or that of non-lymphatic origin.^{317,335} The 24 patients in the 'control group' did not show any evidence of lymphoedema and had normal lymphoscintigraphy, despite the majority demonstrating swollen lower limbs. This confirms that patients can have swollen limbs for other reasons, and that lymphoscintigraphy is important to confirm a normal lymphatic system. Several of the control group ($n = 13$) were diagnosed with lipoedema, which is one of the differential diagnoses of lymphoedema. Lipoedema is a genetic disorder characterised by abnormal deposition of subcutaneous fat in the lower limbs, often with associated mild oedema and in the early stages lymphatic function is normal.^{295,336}

Patients with normal lymphatic anatomy and function should show symmetrical transport through lymphatic vessels and proximal lymph node uptake. All patients in the BCRL and non-BCRL groups who had abnormal images demonstrated abnormalities that are deemed pathognomonic of abnormal lymphatic function. A total of 6/30 patients (20%) showed uptake in popliteal nodes, indicating lymph re-routing through the deep system, raising the possibility of longer duration of lymphatic dysfunction.³³⁴ Dermal backflow is another indicator of abnormal lymphatics, which occurs when lymph re-routes through the skin. A recent study investigating lymphoedematous lower limbs has shown a strong correlation between popliteal node visualisation and dermal backflow.³³⁴ None of the patients in our study demonstrated dermal backflow, which is perhaps due to the fact that

these patients did not demonstrate any swelling in the subcutis of the lower limbs, which is where this abnormality would present itself.

It was only possible to include the removal rate clearance of 14 patients, but there did not appear to be any differences in the clearance patterns either between BCRL and non-BCRL patients or between patients with normal and abnormal scans. As counts were only measured at 3 time-points over 150 min there was a larger potential for error compared to imaging at more frequent time points. However, it was not possible to explain why the counts were increasing in number, despite keeping the methodology consistent for all patients. It is clear that radioactivity cannot increase in amount. Therefore, these spurious results therefore must reflect an artefact, the cause of which has not yet been identified.

Despite this, previous studies have also shown that clearance of tracer from the depot site is not a reliable method for diagnosing lymphoedema of the lower limb.^{326,337}

Quantification, which is the uptake in the lymph nodes expressed as a percentage of the injected depot, is thought to be a more reliable method for diagnosing lymphoedema. Mostbeck *et al* assessed quantification after subcutaneous injection of ^{99m}Tc-Nanocoll in 25 healthy patients and 12 patients with lower limb lymphoedema. They found significantly lower quantification in lymphoedematous legs compared with normal legs ($2.0 \pm 2.5\%$ vs. $14.3 \pm 4.2\%$, $p < 0.001$).³³⁷ A recent study noted that in the presence of unilateral lymphoedema, the contralateral limb

was often found to be abnormal, highlighting the possibility of a pre-existing constitutional weakness of the lymphatics.²⁹⁵ The quantification in this current study showed there was a significant difference in ilio-inguinal activity at 150 min, with patients in the BCRL group showing significantly lower activity when compared to the non-BCRL group, despite not demonstrating lymphoedema of the lower limb. These results support the hypothesis of a constitutional abnormality in patients who develop BCRL. On reflection, this study did not correct for depth of the ilio-inguinal nodes and their distance from the camera head. Although these patients were matched with regard to BMI, thereby minimising this error, a more accurate method of quantification should include posterior images in the analysis to allow calculation of the geometric mean, which would remove this error altogether. Accurate quantification results would have strengthened the diagnosis of normal or abnormal scans by providing quantitative results in addition to the qualitative results of lymphoscintigraphic images. Nevertheless, the number of scans found to be abnormal would have remained the same or even increased in number had quantification been taken into account as a criterion of abnormality on imaging.

This study has shown that a significant number of patients who had previously undergone treatment for breast cancer had abnormal lower limb lymphoscintigraphy irrespective of whether they developed upper limb BCRL or not. This was an unexpected and novel finding. Almost all the breast cancer patients in this study, either with or without BCRL, had systemic therapy in the form of endocrine therapy or chemotherapy. The patients in this study had large tumours

and were heavily node positive, which would explain why so many required aggressive treatment in the form of chemotherapy. Taxanes (paclitaxel and docetaxel) have emerged as important newer chemotherapeutic drugs in the treatment of patients with breast cancer. Early clinical trials involving docetaxel noticed a progressive development of peripheral oedema and non-malignant effusions, which were severe enough to warrant discontinuation of therapy.³³⁸⁻³⁴¹ A suggested mechanism of action is that repeated docetaxel exposure induces endothelial inflammation leading to abnormal capillary permeability.^{208,342} Studies into the mechanism of the development of oedema in patients receiving taxanes have been conducted with capillaroscopy and capillary filtration tests using ^{99m}Tc-albumin and have concluded that there is an abnormality in the capillary permeability and also progressive accumulation of proteins in the interstitial space.²⁰⁸ A study using the wick and wick-in-needle method to assess transcapillary forces also confirmed treatment-induced capillary protein leakage.³⁴² Although these studies are specifically looking at oedema rather than lymphoedema, it is apparent that these agents cause a systemic disruption, which can have a long-lasting effect on the lymphatics. There have been studies linking systemic chemotherapy to BCRL, although the mechanism for this remains unclear.^{206,343-345} A prospective analysis of BCRL in early breast cancer patients undergoing concomitant post-operative radiotherapy and anthracycline-based chemotherapy +/- taxanes found an incidence of BCRL of 44% in the group receiving taxanes, three times higher than the non-taxane group.²⁰⁷ Several patients in this current study also had taxanes as part of their chemotherapy regimen and it could be that BCRL

was precipitated in susceptible patients exposed to such chemotherapy agents. However, such drug-induced changes may not fully explain the high prevalence of abnormal lymphoscintigraphy. Moreover, none of the breast cancer patients displayed evidence of lower limb swelling.

5.10 Conclusion

In summary, this study has shown that a large proportion of breast cancer patients have abnormal lymphatics. The hypothesis was that patients who develop BCRL have abnormal lower limb lymphatics, indicating a global problem with lymphatic function. This was reflected in the quantification results. Although the majority of patients with BCRL did demonstrate abnormal lymphatics, there were also several patients without BCRL who had abnormal lymphatic function, which cannot be fully explained by this hypothesis. One distinct possibility is that it is systemic therapy causing abnormalities of lymphatic function, even though there was no lower limb swelling. There is also the possibility that there is an unidentified association between axillary lymph node metastases, or even breast cancer itself, and lymphatic dysfunction.

	<i>k</i> (%/min) R lower limb	<i>k</i> (%/min) L lower limb	Normal or Abnormal scan
100B	0.1777	0.1058	Abnormal
101B	0.2080	0.1504	Abnormal
102B	0.0811	0.2165	Abnormal
103B	0.1587	0.0807	Normal
104B	0.1387	0.1956	Normal
105B	0.0911	0.0849	Abnormal
100G	0.0555	0.1461	Abnormal
109G	0.0508	0.0344	Normal
Mean ± SD	0.120 ± 0.06	0.127 ± 0.06	

Table 30 Depot clearance *k* (%/min) in lower limbs of patients with BCRL (*n* = 8)

	<i>k</i> (%/min) R lower limb	<i>k</i> (%/min) L lower limb	Normal or Abnormal scan
105G	0.0915	0.1265	Normal
112G	0.0544	0.1439	Normal
116G	0.1772	0.3693	Abnormal
117G	0.1194	0.1771	Abnormal
120G	0.1459	0.1809	Normal
123G	0.0268	0.0690	Abnormal
Mean ± SD	0.103 ± 0.06	0.178 ± 0.10	

Table 31 Depot clearance *k* (%/min) in lower limbs of non-BCRL patients (*n* = 6)

<i>k</i> average (%/min)	
BCRL patients (<i>n</i> = 16)	0.124 ± 0.06
Non-BCRL patients (<i>n</i> = 12)	0.140 ± 0.09
<i>P</i> *	0.58

Table 32 Average *k* (%/min) values when comparing both limbs of BCRL and non-BCRL patients (mean ± SD)

*unpaired *t* test between normal and abnormal scans

	<i>k</i> average (%/min)
Normal scan (<i>n</i> = 12)	0.117 ± 0.05
Abnormal scan (<i>n</i> = 16)	0.141 ± 0.08
<i>p</i>*	0.36

Table 33 *k* (%/min) values for both limbs when comparing normal and abnormal scans (mean ± SD)

*unpaired *t* test between normal and abnormal scans

	<i>k</i> (%/min) right lower limb	<i>k</i> (%/min) left lower limb
103B*	0.1587	0.0807
104B*	0.1387	0.1956
105G	0.0915	0.1265
109G*	0.0508	0.0344
112G	0.0544	0.1439
120G	0.1459	0.1809
Mean ± SD	0.107 ± 0.05	0.127 ± 0.06

Table 34 Depot clearance *k* (%/min) in lower limbs of patients with normal scans (*n* = 8)

*patients with BCRL

	<i>k</i> (%/min) right lower limb	<i>k</i> (%/min) left lower limb
100B*	0.1777	0.1058
101B*	0.2080	0.1504
102B*	0.0811	0.2165
105B*	0.0911	0.0849
100G*	0.0555	0.1461
117G	0.1194	0.1771
120G	0.1459	0.1809
123G	0.0268	0.0690
Mean ± SD	0.117 ± 0.06	0.165 ± 0.10

Table 35 Depot clearance *k* (%/min) in lower limbs of patients with abnormal scans (*n* = 8) *patients with BCRL

	Ilio-inguinal nodal activity as % of depot injection				Normal (N) or abnormal (A) lymphoscintigraphy
	<u>45 minute quantification</u>		<u>150 minute quantification</u>		
	<i>Right lower limb</i>	<i>Left lower limb</i>	<i>Right lower limb</i>	<i>Left lower limb</i>	
100G	0.4	0.1	3.7	0.2	A
102G	0	0.1	0.4	3.6	A
106G	0.2	0.1	0.8	0.8	A
107G	1.2	0.8	3.4	3.9	N
108G	0.1	0	4.2	3.3	A
109G	0.3	0.2	0.8	1.5	N
110G	0.5	1.8	0.7	2.6	A
115G	0.5	5.7	2.8	10.6	N
Mean ± SD	0.4 ± 0.4	1.1 ± 2.0	2.1 ± 1.6	3.3 ± 3.2	

Table 36 Ilio-inguinal nodal activity as a percentage of depot injection at 45 and 150 min in BCRL patients

	Ilio-inguinal nodal activity as % of depot injection				Normal (N) or abnormal (A) lymphoscintigraphy
	<u>45 minute quantification</u>		<u>150 minute quantification</u>		
	<i>Right lower limb</i>	<i>Left lower limb</i>	<i>Right lower limb</i>	<i>Left lower limb</i>	
101G	0.1	0.2	0.3	0.5	N
104G	0	0	1.6	1.2	A
105G	0.1	0.4	6	9.3	N
112G	2	4.9	3.5	7.3	N
113G	0.5	0.2	0.4	2.1	A
114G	0	0	10.1	4.5	A
116G	0.8	2.1	5.1	7.6	A
117G	0.7	3	4.5	7.9	A
118G	0	0	23.3	8.4	A
119G	0.2	1	0.8	2.5	N
120G	0.1	0.6	11	10.4	N
121G	0.4	0.2	5.5	4.3	N
122G	2.7	1.9	10.6	7.5	N
123G	1	0.5	3.7	4.8	A
Mean ± SD	0.6 ± 0.8	1.1 ± 1.4	6.2 ± 6.1	5.6 ± 3.2	

Table 37 Ilio-inguinal nodal activity as a percentage of depot injection at 45 and 150 min in non-BCRL patients

	Quantification 45 min (Ilio-inguinal activity as % of depot injection)	Quantification 150 min
BCRL patients ($n = 16$)	0.75 ± 1.4	2.7 ± 2.5
Non-BCRL patients ($n = 28$)	0.84 ± 1.2	5.9 ± 4.8
<i>p</i>	0.82	0.006

Table 38 Average ilio-inguinal nodal activity as percentage of depot injection for each lower limb at 45 and 150 min for BCRL ($n = 16$) and non-BCRL ($n = 28$) patients

Summary and Conclusion

The aim of the studies in this thesis was to further understand the pathophysiology of BCRL. The traditional view has been that the removal of axillary nodes leads to obstruction of lymph flow in the upper limb, which causes the accumulation of lymph in the interstitium. Previous observations have indicated that the mechanism is more complex than this simple stopcock hypothesis. The investigations in this thesis concentrated on the hypothesis that there may be a constitutive predisposition to BCRL.

The first study investigated the muscle lymph flow in the upper limb of women undergoing surgery for breast cancer. The lymphatic clearance rate was measured to see if there was an abnormality in the lymph flow prior to axillary lymph node surgery. This would pose a constitutional risk for the development of BCRL. Secondly, patients were assessed for the presence of upper limb lymphovenous communications with a view to establishing if these act as a protective mechanism against BCRL. Lastly, the lymphatic system of the lower limbs in patients previously treated for breast cancer was assessed. This was performed with the aim of determining whether there was a disturbance in lymphatic function in patients who had BCRL compared to those without.

Study 1: An investigation into the muscle lymph drainage of the upper limb

In a previous study investigating forearm muscle lymph flow at time intervals after breast cancer surgery it was found that women who went on to develop BCRL had a higher lymph flow rate than non-BCRL patients, reflecting a high rate of capillary fluid filtration, describing this as a 'high filterer' hypothesis. It was not possible to ascertain if this finding existed prior to surgery or was a response to axillary lymph node surgery. The aim of this study was to address this distinction. The main findings were as follows:

- There was a significantly higher mean k in patients who went on to develop BCRL compared with non-BCRL patients. This indicated a constitutional difference in the fluid turnover rather than a response to surgery.
- At 8 weeks post-surgery, there was no major change in muscle lymph drainage, which would be expected if there were truly a stopcock effect.
- Measurement of the axillary activity pre- and post-operatively showed no significant change in activity, indicating that lymphatics and lymph flow remain active after surgery. This is also contrary to the theory of lymphostasis, which is postulated by the stopcock hypothesis.

There was a significant side-to-side correlation of k , reinforcing evidence that quantitative lymphoscintigraphy produces a reproducible measure of lymph drainage, and further validating the use of the contralateral arm as a control. The high fluid filtration could be promoting an imbalance between lymph drainage and fluid filtration thereby predisposing these patients to failure of lymphatic function.

Study 2: An investigation into the presence or absence of lymphovenous communications in the upper limb in breast cancer patients

Previous studies have shown the presence of lymphovenous communications (LVCs) in patients, although the significance remains unknown. The aim of this study was to investigate the presence or absence of LVCs in breast cancer patients and to see if this correlated with the development of BCRL.

The key findings were as follows:

- There was clear evidence of shunting of labelled erythrocytes in several breast cancer patients.
- When shunting was present, it was more marked in patients who did not develop BCRL.

Whilst this study did confirm the presence of LVCs in women undergoing surgery for breast cancer, it could not determine for certain whether LVCs opened up in response to surgery, thereby making it difficult to confirm or refute the hypothesis that LVCs protect against the development of BCRL.

Study 3: An investigation into a constitutional global lymphatic dysfunction in patients with BCRL

Studies have found abnormalities in the lymphatic vessels of the contralateral, non-swollen upper limbs of patients who developed BCRL in addition to abnormalities in the ipsilateral limb. These findings have contributed to the hypothesis of a predisposition to BCRL, which would affect the global lymphatic system. Therefore,

lower limb lymphatic function was studied, which allowed comparison of patients with and without BCRL. The main findings were:

- Patients with BCRL and clinically normal lower limbs showed significant reduction in lower limb ilio-inguinal nodal activity, which was reflected in the significant difference in quantification when compared to non-BCRL patients. This suggested impaired lymph transport in their lower limbs in comparison with those without BCRL.
- Several patients with BCRL were found to have abnormal lower limb lymphoscintigraphy, but an unexpected and intriguing finding was that there were a number of patients without BCRL who also had abnormal lower limb lymphoscintigraphy.

The finding of a high prevalence of abnormal scans in all breast cancer patients has not been reported previously, and images indicate lymphatic dysfunction in the absence of clinical lower limb lymphoedema. It was noted that the vast majority of breast cancer patients studied had undergone systemic therapy as part of their breast cancer treatment, and it has raised the question as to whether this treatment is a contributory factor for this unpredicted observation. A combination of constitutive predisposition and systemic therapy, particularly with the use of taxanes, could contribute to the observed abnormality of lymphatic function. Another possibility is that there is an unidentified association between axillary metastases or breast cancer and lymphatic dysfunction.

Conclusion

The work described in this thesis has demonstrated that the pathophysiology of BCRL is complex and cannot be adequately explained by a simple stopcock hypothesis. On the contrary, the results have shown that the development of BCRL may be inevitable in some patients and secondary to an inherent predisposition. This constitutional susceptibility, in conjunction with systemic breast cancer treatment, could explain why some patients continue to develop BCRL despite the use of better locoregional and systemic therapies. Greater focus on the contribution of genetic predilection to BCRL may be the key to help identify those patients at a higher risk of developing the condition, with a view to introducing better preventative measures and earlier intervention to minimise the consequences of this incurable condition.

Future Work

It is uncertain from these studies whether LVCs pre-exist constitutionally or develop in response to surgery. Future work need not necessarily be based on labelled red cells but perhaps instead on a less labour-intensive method using other labelled particles, such as engineered liposomes. These can be labelled with stable particles and perhaps be combined with MRI scanning to look at the axilla pre- and post-surgery to assess delivery to lymphatics and response to surgery.

Genetic susceptibility is an area that is receiving more interest and future work should focus on biomarkers, which could help identify individuals who are more at risk of developing BCRL.

The unexpected finding of abnormal lower limb lymphatics in patients with and without BCRL has raised the possibility of systemic breast cancer treatment or the susceptibility to breast cancer contributing to this finding. Future work should aim to assess these associations further.

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